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تحاليل طبية**

**Parathormone, Calcium and Phosphorus Levels in Hemodialysis
Patients at Al-Shifa Hospital, Gaza-Palestine**

Prepared by :

Hosam M. Abo Shamala

Supervisor

Prof. Mohammad E. Shubair

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Declaration

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Signature: Hosam

Name: Hosam M. Abo Shamala

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Parathormone, Calcium and Phosphorus Levels in Hemodialysis Patients at Al-Shifa Hospital, Gaza-Palestine

Abstract

Many people who have severe chronic kidney disease (CKD) will eventually develop kidney failure and will require dialysis.

The Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines for bone metabolism and disease in CKD (USA) recommend that, in stage 5 CKD, the target levels for calcium (Ca) (corrected for serum albumin), phosphorus (P), calcium \times phosphorus (Ca \times P) product and parathormone (PTH) levels should be maintained at 8.4-9.5 mg/dl, 3.5-5.5 mg/dl, $< 55 \text{ mg}^2/\text{dl}^2$ and 150-300 pg/ml, respectively.

This study aimed to assess the levels of the previously mentioned parameters in the hemodialysis (HD) patients who have been on HD for ≥ 12 months in the HD unit at Al-Shifa hospital, and comparing the results obtained with that recommended by KDOQI guidelines and with the results of a healthy control group.

Eighty HD patients (cases) (41 males, 39 females; mean age 47.2 ± 15.9 years), on HD for (mean \pm SD: 49.1 ± 38 months, range: 12-163 months), with mean HD frequency (2.6 ± 0.5 sessions weekly), were enrolled in the study. Age and sex matched healthy control subjects were included in the study.

Data were collected through a self constructed structured questionnaire and from biochemical analysis of serum calcium, albumin, phosphorus, PTH, ionized calcium, urea and creatinine of both case and control groups.

It was shown that 58.7%, 77.5%, 67.5% and 86.2% among the cases were out of the target ranges for albumin-corrected serum calcium, phosphorus, calcium \times phosphorus product and PTH, respectively.

There were statistically significant differences in the mean levels of serum PTH, calcium \times phosphorus product, albumin, phosphorus and ionized calcium between cases and controls as follows: (PTH: 1715.3 ± 1706.3 vs 35.7 ± 14.7 pg/ml), (Ca \times P product: 62.7 ± 14.6 vs $40.2 \pm 6.0 \text{ mg}^2/\text{dl}^2$), (albumin: 4.6 ± 0.39 vs 4.7 ± 0.3 g/dl), (P: 6.6 ± 1.4 vs 4.3 ± 0.6 mg/dl) and (ionized calcium: 3.78 ± 0.47 vs 4.7 ± 0.1 mg/dl).

On the other hand, there was no statistically significant difference in the mean levels of albumin-corrected serum calcium between cases and controls (9.5 ± 0.9 vs 9.4 ± 0.3 mg/dl).

Moreover there was a statistically significant positive correlation between serum PTH with HD duration.

There were no statistically significant differences in the mean levels of serum PTH, P, $\text{Ca} \times \text{P}$ product and corrected calcium between cases on HD for 2 sessions weekly and those on HD for 3 sessions weekly. The mean levels of all the mentioned parameters were out of the target range, except for albumin-corrected serum calcium which was in the target range. Also, the same results were obtained in the cases who were receiving vitamin D analogue (alfacalcidol) and those not receiving. It was noted that albumin-corrected serum calcium levels were close to the target range.

There was a statistically significant correlation between the mean of serum creatinine with $\text{Ca} \times \text{P}$ product or with serum P among cases.

The study revealed that there was a statistically significant relationship between the duration of HD and bone diseases.

The study revealed that there was a statistically significant difference in the percentage of cases with cardiovascular disease on HD for 2 sessions weekly (6.5%) and that of cases on HD for 3 sessions weekly (32.7%).

It is recommended that new strategies must be implemented to prevent parathyroid gland hyperplasia and to avoid the positive balance of calcium and phosphorus in the hemodialysis patients.

Key words: Hemodialysis, Phosphorus, Parathyroid gland hyperplasia, Vitamin D analogues, Gaza.

مستويات هرمون الغدد جارات الغدة الدرقية، الكالسيوم و الفوسفور في مرضى غسيل الدم

(الديال الدموى) فى مستشفى الشفاء-غزة- فلسطين

الملخص

إن عدد كبير ممن يشتكون من أمراض الكلى المزمنة الشديدة سيصابون بالفشل الكلوى الذى يتطلب إجراء عملية الديال الدموى.

طبقاً لمبادرة المؤسسة القومية الأمريكية و الخاصة بضبط الجودة لمرض الكلية المزمن و ايض العظام، فإنه فى المرحلة الخامسة من امراض الكلى المزمنة يجب ان يكون مستوى الكالسيوم (مصححاً لقيمة زلال الدم) ، الفوسفور ناتج ضرب الكالسيوم × الفوسفور و هرمون الغدد جارات الغدة الدرقية كالتالى : 8.4-9.5 مج/100مل، 3.5-5.5 مج/100مل ، اقل من 55 مج/2مل و 150-300 بيكوجرام/مل على التوالى.

كان هدف الدراسة هو قياس مستوى المواد المذكورة سابقاً فى مرضى الديال الدموى الذين مر عليهم 12 شهر أو اكثر فى وحدة الديال الدموى بمستشفى الشفاء ومقارنة النتائج مع القيم المذكورة سابقاً وكذلك مع نتائج العينة الضابطة.

شارك فى الدراسة 80 مريض (41 ذكر ، 39 أنثى) وكان متوسط اعمارهم 15.9 ± 47.2 سنة) ومتوسط فترة الديال الدموى (38 ± 49.1 شهر) وبمعدل (0.5 ± 2.6 جلسة /اسبوع) كما شارك فى الدراسة 80 حالة ضابطة متوافقين فى العمر والجنس مع المرضى. تم جمع المعلومات عن طريق استبيان من قبل الباحث وعن طريق التحاليل الكيميائية الحيوية للمرضى والعينة الضابطة.

اظهرت النتائج ان 58.7% و 77.5% و 67.5% و 86.2% من المرضى كانوا خارج المعدل المطلوب لكل من الكالسيوم المصحح و الفوسفور و ناتج ضرب الكالسيوم × الفوسفور و هرمون الغدد جارات الغدة الدرقية على التوالى.

كان هناك فروق ذات دلالات إحصائية فى متوسط مستويات كل من هرمون الغدد جارات الغدة الدرقية و ناتج ضرب الكالسيوم × الفوسفور و الزلال و الفوسفور و الكالسيوم المتأين بين المرضى والعينة الضابطة وكانت النتائج كالتالى : 1706.3 ± 1715.3 مقابل 14.7 ± 35.7 بيكوجرام/مل، (14.6 ± 62.7 مقابل 6.0 ± 40.2 مج/2مل) 0.39 ± 4.6 مقابل 0.3 ± 4.7 جم/100مل، (1.4 ± 6.6 مقابل 0.6 ± 4.3 مج/100مل) و (0.47 ± 3.78 مقابل 0.1 ± 4.7 مج/100مل) على التوالى.

لم يكن هناك فرق ذو دلالة إحصائية فى متوسط مستوى الكالسيوم المصحح بين المرضى والعينة الضابطة (0.9 ± 9.5 مقابل 0.3 ± 9.4 مج/100مل).

كان هناك علاقة ذات دلالة إحصائية بين مستوى هرمون الغدد جارات الغدة الدرقية ومدة الديال الدموى.

لم يكن هناك فرق ذو دلالة إحصائية بين من يغسلون الدم مرتين ومن يغسلونه ثلاث مرات إسبوعياً ، ولا بين من يتناولون نظير فيتامين د (الفاكالسيدول) ومن لا يتناولونه بالنسبة لكل من هرمون الغدد جارات الغدة الدرقية و الفوسفور و ناتج ضرب الكالسيوم \times الفوسفور و الكالسيوم المصحح. كانت كل القيم خارج المعدل المطلوب ماعدا الكالسيوم المصحح الذى كان فى المعدل المطلوب فى الحالة الأولى وقريب من المعدل فى الحالة الثانية.

كان هناك علاقة ذات دلالة إحصائية بين مستوى الكرياتينين مع مستوى ناتج ضرب الكالسيوم \times الفوسفور وكذلك مع مستوى الفوسفور فى مرضى الديال الدموى.

اظهرت الدراسة ان هناك علاقة ذات دلالة إحصائية بين مدة الديال الدموى وامراض العظام. كما اظهرت الدراسة ان هناك فرق ذو دلالة إحصائية بين نسبة المصابين بامراض القلب والأوعية الدموية الذين يغسلون الدم مرتين اسبوعياً (6.5%) والذين يغسلونه ثلاث مرات اسبوعياً (32.7%).

من أهم التوصيات فى هذه الدراسة هى انه يجب تطبيق سياسات جديدة لمنع تضخم الغدد جارات الغدة الدرقية ولتجنب زيادة الكالسيوم والفوسفور فى مرضى الديال الدموى.

الكلمات المفتاحية:

الديال الدموى، الفوسفور، تضخم الغدد جارات الغدة الدرقية، نظائر فيتامين د ، غرة.

Dedication

To my fathers' pure spirit,

to my beloved mother,

to my wife,

to my daughters

Amani, Nour and Dena,

to my brothers

and

to my sisters

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All praises and glory are due to ALLAH for all the bounty and support granted to me.

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Table of contents

Contents	page
Declaration	I
Copy right	II
English abstract	III
Arabic abstract	V
Dedication	VII
Acknowledgment	VIII
Table of contents	IX
List of tables	XII
List of figures	XIII
List of abbreviations	XIV

Chapter 1 Introduction

Introduction	1
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Chapter 2 Literature review

2.1 Minerals	5
2.1.1 Calcium	5
2.1.2 Phosphorus	6
2.2 Parathyroid glands	7
2.2.1 Parathormone	7
2.2.1.1 Parathormone biosynthesis	7
2.2.1.2 Actions of parathormone	8
2.2.1.3 Regulation of parathormone gene	11
2.2.1.4 Peripheral metabolism of parathormone	12
2.2.1.5 Hyperparathyroidism	12
2.2.1.6 Hypoparathyroidism	13
2.3 Vitamin D	13
2.3.1 Metabolism of vitamin D	13
2.3.2 Mechanism of action of vitamin D	15
2.3.3 Vitamin D deficiency	16
2.3.4 Consequences of vitamin D deficiency	16
2.3.5 Vitamin D analogues	16
2.4 The kidneys	17
2.4.1 Excretory function of the kidney	19
2.4.2 Other functions of the kidney	20
2.4.3 Kidney disease	20
2.4.4 Stages of CKD	20
2.5 Hemodialysis	21
2.5.1 Epidemiology and etiology	21
2.5.2 Criteria for placing patients on dialysis	22
2.5.3 Medical management of hemodialysis patients	22
2.6 Related studies	24

Chapter 3 Materials and Methods

3.1 Study design	26
3.2 Target population	26
3.3 Setting of the study	26
3.4 Sample size	26
3.5 Ethical considerations	26
3.6 Data collection	26
3.7 Determination of BMI	27
3.8 Blood sampling and processing	27
3.9 Biochemical analysis	27
3.9.1 Determination of serum urea	28
3.9.2 Determination of serum creatinine	29
3.9.3 Determination of serum albumin	30
3.9.4 Determination of serum calcium	31
3.9.5 Determination of serum phosphorus	33
3.9.6 Determination of serum parathormone	34
3.9.7 Determination of serum free- ionized calcium	37
3.10 Statistical analysis	37

Chapter 4 Results

4.1 Study population description	38
4.2 Relationship between parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus with gender among cases & controls	42
4.3 Levels of parathormone, calcium \times phosphorus product, serum albumin, albumin-corrected serum calcium and serum phosphorus among cases & controls	44
4.4 Relationship between parathormone with albumin-corrected serum calcium and with serum phosphorus among cases	47
4.5 Relationship between vitamin D analogue (alfacalcidol), with parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus among cases.	47
4.6 Relationship between parathormone and calcium \times phosphorus product with serum urea and creatinine among cases.	48
4.7 Relationship between parathormone and calcium \times phosphorus product with age of cases.	50
4.8 Relationship between parathormone and calcium \times phosphorus product with BMI of cases.	50
4.9 Relationship of hemodialysis with bone and cardiovascular diseases among cases	50

Chapter 5 Discussion

5.1 Relationship between parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus with gender among cases & controls.	53
5.2 Levels of parathormone, calcium \times phosphorus product, serum albumin, albumin-corrected serum calcium and serum phosphorus among cases & controls	54

5.3 Averages of parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus among cases by duration of hemodialysis.	56
5.4 Averages of parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus among cases by frequency of hemodialysis.	57
5.5 Relationship between parathormone with albumin-corrected serum calcium and with serum phosphorus	57
5.6 Relationship between vitamin D analogue (alfacalcidol), with parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus among cases.	58
5.7 Relationship between parathormone and calcium \times phosphorus product with serum urea and creatinine among cases	59
5.8 Relationship between parathormone and calcium \times phosphorus product with age of cases.	60
5.9 Relationship between parathormone and calcium \times phosphorus product with BMI of cases	60
5.10 Relationship of hemodialysis with bone and cardiovascular diseases among cases	61
Chapter 6 Conclusions and Recommendations	
6.1 Conclusions	63
6.2 Recommendations	64
References	65
Appendices	80

List of Tables

Table (4.1): Averages of parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus among cases by gender	43
Table (4.2): Averages of parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus among controls by gender	43
Table (4.3) Distribution of cases by levels of parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus	44
Table (4.4): Averages of parathormone, calcium \times phosphorus product, serum albumin, albumin-corrected serum calcium, serum free-ionized calcium and serum phosphorus among cases and controls	45
Table (4.5): Averages of parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus among cases by duration of hemodialysis	46
Table (4.6): Averages of parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus among cases by frequency of hemodialysis	47
Table (4.7): Averages of parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus among cases by vitamin D analogue supplying	48

List of Figures

Figure (2.1): Schematic diagram of cutaneous production of vitamin D and its metabolism and regulation for calcium homeostasis and cellular growth.	14
Figure (2.2): Gross anatomy of the kidney	17
Figure (2.3): Nephron anatomy.	19
Figure (4.1): Distribution of study population by gender.	38
Figure (4.2): Distribution of study population by governorates	38
Figure (4.3): Average of weight among cases and controls	39
Figure (4.4): Average of height among cases and controls	39
Figure (4.5): Average of BMI among cases and controls.	40
Figure (4.6): Averages of duration and frequency of hemodialysis among cases	40
Figure (4.7): Distribution of cases by duration of hemodialysis.	41
Figure (4.8): Distribution of cases by frequency of hemodialysis	41
Figure (4.9): Distribution of cases and controls by bone and cardiovascular diseases	42
Figure (4.10): Correlation between $\text{Ca} \times \text{P}$ product with creatinine.	49
Figure (4.11): Correlation between serum phosphorus with creatinine.	49
Figure (4.12): Distribution of cases by bone diseases and duration of hemodialysis.	51
Figure (4.13): Distribution of cases by bone diseases and frequency of hemodialysis	51
Figure (4.14): Distribution of cases by cardiovascular diseases and duration of hemodialysis	52
Figure (4.15): Distribution of cases by cardiovascular diseases and frequency of hemodialysis.	52

List of Abbreviations

AKD	Acute Kidney Disease
BMI	Body Mass Index
Ca	Calcium
CaR	Calcium-Sensing Receptor
CKD	Chronic Kidney Disease
CRF	Chronic Renal Failure
CTAL	Cortical Thick Ascending Limb
DCT	Distal Convoluted Tubule
DOPPS	Dialysis Outcomes and Practice Patterns Study
ESRD	End-Stage Renal Disease
HD	Hemodialysis
KDOQI	The Kidney Disease Outcomes Quality Initiative
M-CSF	Macrophage Colony-Stimulating Factor
NKFKDOQI	The National Kidney Foundation Kidney Disease Outcomes Quality Initiative
OPG	Osteoprotegerin
P	Phosphorus
PCT	Proximal Convoluted Tubule
PTH	Parathormone
RANK	Receptor Activator for Nuclear Factor K B
RANKL	Receptor Activator for Nuclear Factor K B Ligand
VDBP	Vitamin D Binding Protein
VDR	Vitamin D Receptor

Chapter 1

Introduction

The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI)

The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) or KDOQI (USA) provides evidence-based clinical practice guidelines for all stages of chronic kidney disease and related complications. KDOQI expands the Dialysis Outcomes Quality Initiative or DOQI, a project begun by the National Kidney Foundation in 1997 and recognized throughout the world for improving the care of dialysis patients. There are 12 current sets of KDOQI guidelines [1].

K/DOQI guidelines for bone metabolism and disease in chronic kidney disease (CKD) recommend that, in stage 5 CKD, the target levels for calcium (Ca) (corrected for serum albumin), phosphate (P), calcium x phosphate (Ca x P) product and parathyroid hormone (PTH) levels should be maintained at 8.4-9.5mg/dl, 3.5-5.5 mg/dl, $< 55\text{mg}^2/\text{dl}^2$ and 150-300 pg/ml, respectively [2].

Kidney Disease

Kidney disease may be acute or chronic:

1-Acute kidney disease :

Acute kidney disease (AKD) is a syndrome characterized by rapid decline in glomerular filtration rate (hours to days), retention of nitrogenous waste products, and perturbation of extracellular fluid volume and electrolyte and acid-base homeostasis. AKD complicates approximately 5% of hospital admissions and up to 30% of admissions to intensive care units. AKD is usually asymptomatic and diagnosed when biochemical monitoring of hospitalized patients reveals a recent increase in blood urea and creatinine concentrations [3].

2- Chronic kidney disease

Chronic kidney disease is a pathophysiologic process with multiple etiologies, resulting in the inexorable attrition of nephron number and function and frequently leading to end-stage renal disease (ESRD). In turn, ESRD represents a clinical state or condition in which there has been an irreversible loss of endogenous renal function, of a

degree sufficient to render the patient permanently dependent upon renal replacement therapy (dialysis or transplantation) in order to avoid life-threatening uremia [4].

Chronic kidney disease was classified into 5 stages by K/DOQI

- Stage 1: Kidney damage with normal or increased GFR (>90 mL/min/1.73 m²)
- Stage 2: Mild reduction in GFR (60-89 mL/min/1.73 m²)
- Stage 3: Moderate reduction in GFR (30-59 mL/min/1.73 m²)
- Stage 4: Severe reduction in GFR (15-29 mL/min/1.73 m²)
- Stage 5: Kidney failure (GFR <15 mL/min/1.73 m² or dialysis) [5].

Complications of chronic kidney disease

Among the common complications seen in persons with CKD, particularly in those on long-term hemodialysis (HD), is secondary hyperparathyroidism. This affects one in four patients receiving HD [6]. Hypocalcemia is a common condition in chronic renal disease because of declining serum levels of calcitriol. Rising serum phosphorus levels due to impaired phosphorus excretion by the kidneys further contribute to hypocalcemia by lowering serum ionized calcium levels and by inhibiting the action of calcitriol [7].

Renal osteodystrophy in its broadest context encompasses all the disorders of bone and mineral metabolism caused by chronic renal failure (CRF) [8].

CKD is associated with substantially increased risk for cardiovascular disease morbidity and mortality, independent of traditional cardiovascular risk factors such as diabetes, hypertension, lipoprotein levels, and tobacco use [9].

Recently, interest has focused on the roles of hyperphosphatemia, elevated levels of the calcium x phosphorus product and hyperparathyroidism in the development of cardiovascular disease in ESRD. A high prevalence of coronary artery calcification among young adults receiving dialysis, especially those who had been receiving dialysis for more than 10 years, was demonstrated [10].

When conservative management of ESRD is inadequate, HD, peritoneal dialysis, and kidney transplantation are alternatives [11].

Hemodialysis

HD is a medical procedure that uses a special machine (a dialysis machine) to filter waste products from the blood and to restore normal constituents to it. HD is frequently done to treat ESRD. Under such circumstances, kidney dialysis is typically administered using a fixed schedule of three times per week [12].

In Palestine renal failure is one of the most important problems on the healthcare delivery system. As per the year 2000 and 2001 statistics, there were 351 and 400 patients who were maintained on HD and peritoneal dialysis, respectively. The most common causes for end-stage renal failure in Palestine is glomerulonephritis and diabetic nephropathies. The death rate among patients on dialysis is 7-8%; the cardiac and cerebrovascular complications are the main causes of death. The HD services in Palestine were initiated in 1972 [13]. There are 12 working HD centers in Palestine, 8 in West-Bank and 4 in Gaza [14].

According to the annual report of the hospital general administration, the number of the HD patients in Gaza strip, in year 2003 was 204 patients. Currently, there are about 361 patients who are maintained on regular HD in Gaza strip, of them about 180 patients are in the HD unit at Al-Shifa hospital, which have 32 HD machines.

Aim of the study

This study is proposed to assess the levels of PTH, P, albumin-corrected serum Ca, and $(Ca \times P)$ product in patients who are on HD for one year or more in the HD unit at Al-Shifa hospital.

The Specific objectives are:

- 1- To determine the levels of PTH, P, albumin-corrected serum Ca, $(Ca \times P)$ product, and serum free- ionized calcium in both HD patients and apparently healthy control group, and comparing the results of the HD patients with that recommended by KDOQI guidelines.
- 2- To assess a possible relation between PTH, P, albumin-corrected serum Ca, or $(Ca \times P)$ product, with the duration, frequency of HD, or with vitamin D analogue alfacalcidol (1α -hydroxyvitamin D_3) consumption.

- 3- To assess a possible relation between PTH with albumin-corrected serum Ca, or P among cases.
- 4- To assess a possible relation between PTH, or $(Ca \times P)$ product with serum urea, creatinine, age, or body mass index (BMI) among cases.
- 5- To assess a possible relation between HD with bone or cardiovascular diseases.

Significance

The clinical significance of the study is to see the extent to which PTH, Ca, P, and $(Ca \times P)$ product, are maintained in the recommended range of the (K/DOQI) guidelines. An elevated $(Ca \times P)$ product (> 55) increases the risk of metastatic calcification, a complication associated with cardiac dysfunction and death, also sustained over-activity of the parathyroid glands can lead to nodular forms of hyperplasia that are resistant to vitamin D and calcium regulation, are largely irreversible, and typically require parathyroidectomy. Therefore, through early and continuous monitoring of the PTH level in the HD patients they could avoid the parathyroidectomy. This is the first study to be conducted in Gaza according to the knowledge of the researcher.

Chapter 2

Literature review

2.1 Minerals

2.1.1 Calcium

Calcium is the most plentiful cation in the body which contains 25 to 35 mol (1.0 to 1.4 kg) in the adults. Over 98% is in the bones and teeth, the former providing a large reserve which can be drawn on as required [15]. One percent of bone calcium is rapidly exchangeable with extracellular calcium; this calcium is equally distributed between the intracellular and extracellular fluids. Extracellular calcium is the principal substrate for the mineralization of cartilage and bone, but it also serves as a cofactor for many extracellular enzymes, most notably the enzymes of the coagulation cascade [16].

Calcium in serum exists in three fractions: bound to plasma proteins (approximately 40%), chelated to serum anions (13%) and free ionized calcium (47%) [17], and it is this fraction that is biologically active and that is tightly controlled by hormonal mechanisms.

About 1 g calcium is ingested per day of which between about 0.25 to 0.5 g is absorbed. Vitamin D, in the form of 1,25-dihydroxy-cholecalciferol, is needed for adequate calcium absorption. Calcium is lost in urine and faeces. Calcium in the intestine may be rendered insoluble by complexing with large amounts of phosphate or fatty acids and so cannot be absorbed. Oral phosphate may be used therapeutically to reduce calcium absorption [18]. The kidneys reabsorb up to 98% of filtered calcium, with <2% excreted in the urine. Patients with ESRD initiating HD are usually hypocalcemic [19].

Variations in the concentration of ionized calcium in blood modulate the minute-to-minute release of PTH in vivo by affecting the level of activation of the calcium-sensing receptor (CaR) in parathyroid cells [20]. Hypocalcemia stimulates PTH release directly by inactivating the CaR, and plasma PTH concentrations increase within minutes as the concentration of blood ionized calcium declines [21].

Reductions in extracellular calcium concentration that persist for several hours also enhance pre-pro-PTH gene transcription, ultimately making more hormone available for secretion [22]. Sustained periods of hypocalcemia lasting days, weeks or

months promote the development of parathyroid gland hyperplasia, a prominent feature of secondary hyperparathyroidism caused by CRF that markedly increases the mass of parathyroid tissue available for PTH synthesis and secretion [23].

2.1.2 Phosphorus

Phosphorus constitutes approximately 1% of a person's total body weight [24], of which 87% is present in bones, the remainder being found in cells and soft tissues [15].

Phosphate is more widely distributed to non-osseous tissues than is calcium. In human serum, inorganic phosphate (Pi) is present at a concentration of approximately 1mM and exists almost entirely in ionized form as either H_2PO_4^- or HPO_4^{--} . Only 12% of serum phosphate is protein – bound, and an additional small fraction is loosely complexed with calcium, magnesium, and other cations [16].

Organic phosphate is a key component of virtually all classes of structural, informational, and effector molecules that are essential for normal genetic, developmental, and physiologic processes. Of particular importance are the high-energy phosphate ester bonds present in molecules such as adenosine triphosphate (ATP), diphosphoglycerate, and creatine phosphate that store chemical energy [16].

Approximately 1400 mg of phosphorus is ingested daily in a normal adult, of which 65% is absorbed. In order to achieve a neutral phosphate balance, excretion of phosphorus must be proportional to the amount ingested, with approximately 35% excreted in the stool and 65% excreted in the urine. This homeostasis unravels in ESRD, and patients develop elevated serum phosphorus levels [19].

The kidney is the primary regulator of plasma phosphate concentration, PTH being the main influence leading to decreased tubular reabsorption with some contribution from 1,25-dihydroxy-cholecalciferol [15].

Hyperphosphatemia promotes the development of parathyroid gland hyperplasia, and high ambient phosphorus concentrations facilitate PTH synthesis by stabilizing PTH mRNA and facilitating message translation [25]. Persistent hyperphosphatemia may also

diminish the effectiveness of treatment with calcitriol in patients with established secondary hyperparathyroidism [26].

Apart from its role as a contributor to hyperparathyroidism, hyperphosphatemia represents an independent risk factor for death in patients treated with HD even after adjusting for other co-morbid conditions [27]. Death from cardiovascular causes largely accounts for the excess mortality [28].

Indeed, strong relationships have been found between cardiac deaths and factors that favor metastatic calcifications (i.e., hyperphosphatemia and increased calcium \times phosphate product) [29].

The importance of the minerals for normal cellular physiology as well as skeletal integrity is reflected in the powerful endocrine control mechanisms that have evolved to maintain their extracellular concentrations within relatively narrow limits.

2.2 Parathyroid glands

The Parathyroid glands are derived from the developing pharyngeal pouches that also give rise to the thymus. They normally lie in close proximity to the upper and lower poles of each thyroid lobe, but they may be found anywhere along the pathway of descent of the pharyngeal pouches, including the carotid sheath and the thymus and elsewhere in the anterior mediastinum. In contrast to several other endocrine glands, the activity of the parathyroids is controlled by the level of free ionized calcium in the blood stream rather than by trophic hormones secreted by the hypothalamus and pituitary. Normally, decreased levels of free calcium stimulate the synthesis and secretion of parathyroid hormone [30].

2.2.1 Parathormone

2.2.1.1 Parathormone biosynthesis

PTH, a protein of 84 amino acids, is synthesized as a larger precursor, pre-pro-parathyroid hormone (pre-pro-PTH). These pre-pro-PTH sequences share a 25-residue(pre) or signal sequence and a 6- residue (pro) sequence. The signal sequence,

along with the short pro sequence, functions to direct the protein into the secretory pathway. During transit across the membrane of the endoplasmic reticulum, the signal sequence is cleaved off and rapidly degraded. The importance of the signal sequence for normal secretion of PTH is illustrated by the hypoparathyroidism inherited in families carrying mutations in the signal sequence of pre-pro-PTH [31]. After cleavage of the pro sequence, the mature PTH (1 – 84) is concentrated in secretory vesicles and granules. One morphologically distinct subtype of granule contains both PTH and the proteases cathepsin B and cathepin H [32]. This co-localization of proteases, and PTH in secretory granules probably explains the observation that a portion of the PTH secreted from parathyroid glands consists of carboxy-terminal PTH fragments [33].

The intracellular degradation of newly synthesized PTH provides an important regulatory mechanism. Under conditions of hypercalcemia, the secretion of PTH is substantially decreased, and most of what is secreted consists of carboxy-terminal fragments [34].

2.2.1.2 Actions of parathormone

A. Actions on the kidney

1-Stimulation of calcium reabsorption

Almost all of the calcium in the initial glomerular filtrate is reabsorbed by the renal tubules. Sixty-five percent is reabsorbed by the proximal convoluted and straight tubules via a passive, paracellular route [35,36].

PTH does little to affect calcium flux in this region. The remaining calcium is largely reabsorbed more distally, 20% of the initial filtrate in the cortical thick ascending limb (cTAL) of Henle's loop and 10% in the distal convoluted and connecting tubules. In the cTAL, calcium reabsorption also is mainly passive and paracellular, although some transcellular, active calcium transport may occur as well [37].

The calcium- sensing receptor, initially characterized in the parathyroid, also is expressed in the cTAL [38].

The primary site for hormonal regulation of renal calcium reabsorption is the distal nephron, which normally reabsorbs nearly all of the remaining 10% of filtered calcium by a unique transcellular active transport mechanism.

To protect the low physiologic level of cytosolic free calcium from the relatively large amounts of incoming calcium, these cells express the calcium-binding protein calbindin-D28K, which avidly binds entering calcium at the apical membrane and transports it to the basolateral membrane, where it is then ejected via active processes involving sodium-calcium exchange and an ATP-driven calcium pump. Expression of calbindin-D28K is increased by PTH directly and also indirectly, via increased synthesis of 1,25-dihydroxy-cholecalciferol [39]. Sodium-calcium exchange also is increased by PTH [40].

2- Inhibition of phosphate transport

Phosphate reabsorption occurs mainly in the proximal renal tubules, which reclaim roughly 80% of the filtered load. Some additional phosphate (8% to 10%) is reabsorbed in the distal tubule (but not in Henle's loop), leaving about 10% to 12% for excretion in the urine. Phosphate reabsorption in both proximal and distal tubules is strongly inhibited by PTH. Phosphate is reabsorbed by a transepithelial route. Transport from the glomerular filtrate into the cell is mediated by specific sodium – (inorganic) phosphate (Na Pi) cotransporters [41]. In response to PTH, the V_{max} for sodium-phosphate cotransport decreases because (Na Pi) cotransporters are rapidly (by 15 minutes) sequestered within subapical endocytic vesicles, after which they are delivered to lysosomes and undergo proteolysis [42]. Conversely, in hypoparathyroidism expression of (Na Pi) protein and mRNA is strongly up-regulated [43].

3-Other renal effects of parathormone

PTH stimulates the synthesis of 1,25-dihydroxy-cholecalciferol (1,25(OH)₂D₃) in the proximal tubule by rapidly inducing transcription of the 25-hydroxy-vitamin D [25(OH)D] 1 α -hydroxylase gene, an effect that can be overridden by hypercalcemia or by the action of 1,25(OH)₂D₃ [44]. PTH also stimulates proximal tubular gluconeogenesis [45].

B. Actions of parathormone on bone

The actions of PTH on bone are complicated because PTH acts on a number of cell types both directly and indirectly. PTH increases both bone formation and bone resorption. With regard to calcium homeostasis, the effect of PTH on bone resorption is dominant; continuous administration of PTH leads to a net release of calcium from bone. But this straightforward net effect of PTH on calcium homeostasis belies the highly variable effects of the hormone on bone, which depend on the type of bone (trabecular or cortical), the particular target cell type, and the pattern of PTH administration [16].

Receptors for PTH are found on preosteoblasts, osteoblasts, lining cells, and osteocytes. PTH changes the osteoblast lineage cell population by stimulating cell proliferation [46], by decreasing apoptosis of preosteoblasts and osteoblasts, thereby increasing the number of osteocytes; and perhaps by converting inactive lining cells to osteoblasts [47].

When added to cells in culture, PTH stops preosteoblastic cells from becoming mature osteoblasts [48]. When PTH is added to isolated osteoblasts *in vitro*, the osteoblasts decrease their synthesis of collagen I and other matrix proteins, at least in part by steering the essential transcription factor core-binding factor- α 1 (CBFA1) toward proteosomal destruction [49].

In vivo, however, the most obvious effects of PTH are to increase bone formation, probably by indirect actions such as stimulation of synthesis of insulin-like growth factor 1 (IGFI) and other growth factors by osteoblast lineage cells. This action of PTH can be mimicked *in vitro* by intermittent administration of PTH to osteoblasts in organ culture [46].

Surprisingly, osteoclasts, the bone-resorbing cells derived from hematopoietic precursors, have no PTH receptors. Instead, preosteoblasts and osteoblasts signal to osteoclast precursors to cause them to fuse and form mature osteoclasts and signal to those osteoclasts to allow them to resorb bone and to avoid apoptosis [16].

Two osteoblast surface proteins, macrophage colony-stimulating factor (M-CSF) and Receptor Activator for Nuclear Factor κ B Ligand (RANKL), are essential for stimulation of osteoclastogenesis [50,51], and RANKL is essential for the activation of mature osteoclasts. The growth factor M-CSF (or CSF1), is expressed both as a secreted protein and as a cell surface protein; the production of both is stimulated by PTH [52]. RANKL is also increased by PTH. RANKL binds to its receptor, RANK which is found both on osteoclast precursors and on mature osteoclasts. The binding of RANKL to RANK can be blocked by osteoprotegerin (OPG), which is secreted by cells of the osteoblastic lineage. PTH decreases the synthesis and secretion of OPG from these cells. Thus, PTH by increasing RANK and decreasing OPG locally in bone, serves to increase bone resorption [53].

Because PTH can both increase bone formation and increase resorption, the net effect of PTH on bone mass varies from one part of bone to another and also varies dramatically according to whether PTH is administered continuously or intermittently. Intermittent administration of low doses of PTH causes dramatic net increase in trabecular bone mass with little effect on cortical bone in humans [16]. Continuous administration of PTH, in contrast, leads to a decrease in cortical bone mass; the net effect of PTH on trabecular bone depends on the dose. In mild hyperparathyroidism, there is little net effect of PTH on trabecular bone and a decrease in cortical bone. In all of these settings, the rate of bone formation is increased; the varying rate of osteoclastic resorption determines the net effect of PTH on bone mass [16].

2.2.1.3 Regulation of the parathormone gene

Although 1,25(OH)₂D₃ – the active form of vitamin D- has no direct effect on PTH secretion, it dramatically suppresses PTH gene transcription [54].

Calcium also regulates the biosynthesis of PTH. *In vivo* studies show that acute hypocalcemia in rats leads, within an hour, to an increase in PTH messenger RNA (mRNA) [55,56]. In contrast, hypercalcemia leads to little [56] or no [55] change in PTH mRNA. The parathyroid gland is poised to respond to a fall in calcium much more readily than to a rise.

The mechanism for the increase in PTH mRNA in response to hypocalcemia is uncertain; differing experimental paradigms suggest regulation at the levels of gene transcription, [57] mRNA stability, [58] and mRNA translation [59].

A series of studies *in vitro* [60], and *in vivo* [61], have demonstrated that phosphate can increase PTH secretion directly, independent of effects on blood calcium and 1,25(OH)₂D₃.

2.2.1.4 Peripheral metabolism of parathormone

Both PTH (1-84) and carboxy-terminal fragments of PTH are secreted from the parathyroid gland. Secreted intact PTH (1-84) is extensively metabolized by liver (70%) and kidney (20%) and disappears from the circulation with a half-life of 2 minutes. Less than 1% of the secreted hormone finds its way to PTH receptors on physiologic target organs [62].

In the liver, a small amount of PTH binds to physiologically relevant PTH receptors but most of the intact PTH is cleaved, initially after residues 33 and 36, probably by cathepsins. [63]. In the kidney, a small amount of intact PTH binds to physiologic PTH receptors, but most of the intact PTH is filtered at the glomerulus and subsequently bound by a large, membrane-bound luminal protein, megalin [64]; this binding leads to internalization and degradation of PTH by the tubules [65].

2.2.1.5 Hyperparathyroidism

Hyperparathyroidism, characterized by excess production of PTH, is a common cause of hypercalcemia and is usually the result of autonomously functioning adenomas or hyperplasia [66]. Hyperparathyroidism occurs in two major forms. Primary and secondary, and less commonly as tertiary hyperparathyroidism.

Primary hyperparathyroidism is usually caused by a parathyroid adenoma or by primary hyperplasia of the glands. On rare occasions (less than 1% of cases), it is caused by a carcinoma of the parathyroids [30]. The manifestations may be subtle, and the disease may have a benign course for many years or a lifetime. This milder form of the disease is usually termed asymptomatic hyperparathyroidism [66].

Secondary hyperparathyroidism is caused by any condition associated with a chronic depression in the serum calcium level, because low serum calcium leads to compensatory overactivity of the parathyroids. Renal failure is by far the most common cause of secondary hyperparathyroidism. Chronic renal insufficiency is associated with hyperphosphatemia. The elevated serum phosphate levels directly depress serum calcium levels and thereby stimulate parathyroid gland activity. In a minority of patients, parathyroid activity may become autonomous and excessive, a process sometimes termed **tertiary hyperparathyroidism**. Parathyroidectomy may be necessary to control the hyperparathyroidism in such patients [30].

2.2.1.6 Hypoparathyroidism

Hypoparathyroidism is far less common than hyperparathyroidism. The major causes of hypoparathyroidism are :

- Surgical ablation : advertent removal of parathyroids during thyroidectomy.
- Congenital absence.
- Autoimmune hypoparathyroidism : a hereditary polyglandular deficiency syndrome arising from autoantibodies to multiple endocrine organs.

The major clinical manifestation of hypoparathyroidism are referable to hypocalcemia[30].

2.3 Vitamin D

Vitamin D is a group of fat-soluble prohormones, the two major forms of which are vitamin D₂ (or ergocalciferol) and vitamin D₃ (or cholecalciferol) [67]. Vitamin D₂ is derived from fungal and plant sources, and is not produced by the human body. Vitamin D₃ is derived from animal sources and is made in the skin when 7-dehydrocholesterol reacts with UVB ultraviolet light at wavelengths between 270–300 nm, with peak synthesis occurring between 295-297 nm [68,69].

2.3.1 Metabolism of vitamin D

During exposure to sunlight, 7-dehydrocholesterol (7-DHC) in the skin absorbs solar UVB radiation and is converted to previtamin D₃ (preD₃). Once formed, previtamin D₃ undergoes thermally induced transformation to vitamin D₃. Additional

exposure to sunlight converts previtamin D₃ and vitamin D₃ to biologically inert photoproducts. Vitamin D originating from the diet or from the skin enters the circulation and is metabolized to 25(OH)D₃ in the liver by vitamin D 25-hydroxylase (25-OHase). 25(OH)D₃ reenters the circulation and is converted to 1,25(OH)₂D₃ in the kidney by 25(OH)D₃ 1 α -hydroxylase (1-OHase). A variety of factors, including serum phosphorus (P_i) and PTH, regulate the renal production of 1,25(OH)₂D. 1,25(OH)₂D regulates calcium metabolism through interactions with its major target tissues, i.e., bone and intestine. 1,25(OH)₂D also induces its own destruction by enhancing the expression of 25(OH)D 24-hydroxylase (24-OHase). 25(OH)D is metabolized in other tissues for regulation of cellular growth [70], as shown in **Figure 2-1**.

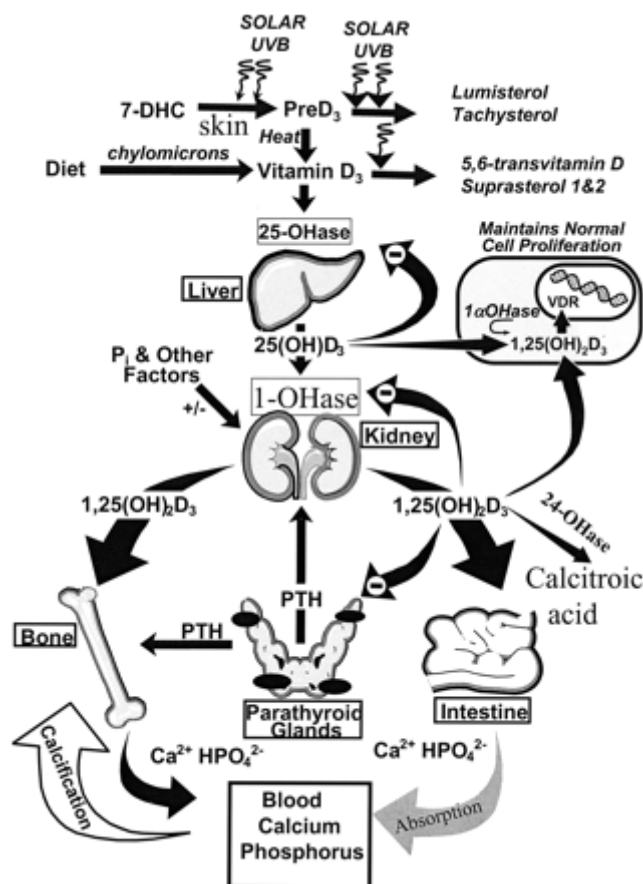


Figure 2-1. Schematic diagram of cutaneous production of vitamin D and its metabolism and regulation for calcium homeostasis and cellular growth [70].

2.3.2 Mechanism of action of vitamin D

Once vitamin D is produced in the skin or consumed in food, it is converted in the liver and kidney to form 1,25 dihydroxyvitamin D, (1,25(OH)₂D). Following this conversion, the hormonally active form of vitamin D is released into the circulation, and by binding to a carrier protein in the plasma, vitamin D binding protein (VDBP), it is transported to various target organs [71].

The hormonally active form of vitamin D mediates its biological effects by binding to the vitamin D receptor (VDR), which is principally located in the nuclei of target cells [71]. The binding of calcitriol (1,25(OH)₂D) to the VDR allows the VDR to act as a transcription factor that modulates the gene expression of transport proteins (such as TRPV6 and calbindin), which are involved in calcium absorption in the intestine. VDR activation in the intestine, bone, kidney, and parathyroid gland cells leads to the maintenance of calcium and phosphorus levels in the blood (with the assistance of parathyroid hormone and calcitonin) and to the maintenance of bone content [70]. The VDR is known to be involved in cell proliferation, differentiation. Vitamin D also affects the immune system, and VDR are expressed in several white blood cells including monocytes and activated T and B cells [72].

The vitamin D receptor acts by forming a heterodimer with the retinoid-X receptor, binding to DNA elements, and recruiting coactivators in a ligand-dependent fashion [73]. Vitamin D plays an important role in the maintenance of organ systems [74].

Vitamin D regulates the calcium and phosphorus levels in the blood by promoting their absorption from food in the intestines, and by promoting re-absorption of calcium in the kidneys. Vitamin D promotes bone formation and mineralization and is essential in the development of an intact and strong skeleton. However, at very high levels it will promote the resorption of bone. It inhibits parathyroid hormone secretion from the parathyroid gland. Vitamin D affects the immune system by promoting phagocytosis, anti-tumor activity, and immunomodulatory functions [75].

2.3.3 Vitamin D deficiency

Vitamin D deficiency can result from inadequate intake coupled with inadequate sunlight exposure, disorders that limit its absorption, conditions that impair conversion of vitamin D into active metabolites, such as liver or kidney disorders, or, rarely, by a number of hereditary disorders [74].

2.3.4 Consequences of vitamin D deficiency

Vitamin D deficiency is known to cause several bone diseases including: rickets, a childhood disease characterized by impeded growth, and deformity, of the long bones. Osteomalacia, a bone-thinning disorder that occurs exclusively in adults and is characterized by proximal muscle weakness and bone fragility.

Osteoporosis, a condition characterized by reduced bone mineral density and increased bone fragility [76].

Vitamin D malnutrition may also be linked to an increased susceptibility to several chronic diseases such as high blood pressure, tuberculosis, cancer, periodontal disease, multiple sclerosis, chronic pain, depression, schizophrenia, seasonal affective disorder, peripheral artery disease [77], and several autoimmune diseases including type1 diabetes [70,78].

2.3.5 Vitamin D analogues

The recognition that 1,25(OH)₂D₃ promotes cellular differentiation and inhibits cellular proliferation has led to efforts directed at producing new analogues that retain these effects but do not cause hypercalcemia [16] These analogues are relatively selective for parathyroid gland with lesser effect on intestinal absorption of calcium and phosphorus. **22-oxacalcitriol** is characterized by decreasing affinity for vitamin D binding protein and has a short plasma half-life resulting in rapid clearance from the circulation. It is also characterized by effectively decreasing PTH secretion without affecting bone turnover. **Paricalcitol** is characterized by adequately controlling PTH secretion with minimal hypercalcemia and hyperphosphatemia compared to calcitriol. Also, **doxercalciferol** has the same effective suppression of PTH with minimal changes in serum calcium and phosphorus levels [79].

2.4 The kidneys

The kidneys are small, dark red organs lie against the dorsal body wall in a retroperitoneal position. They receive some protection from the lower part of the rib cage. An adult kidney is about 12 cm long, 6 cm wide, and 3 cm thick [80]. The kidney has three regions **Figure 2-2**. The **renal cortex** is an outer, granulated layer, the **renal medulla** consists of cone-shaped tissue masses called renal pyramids, and the **renal pelvis** which is a central space, or cavity, that is continuous with the ureter [81].

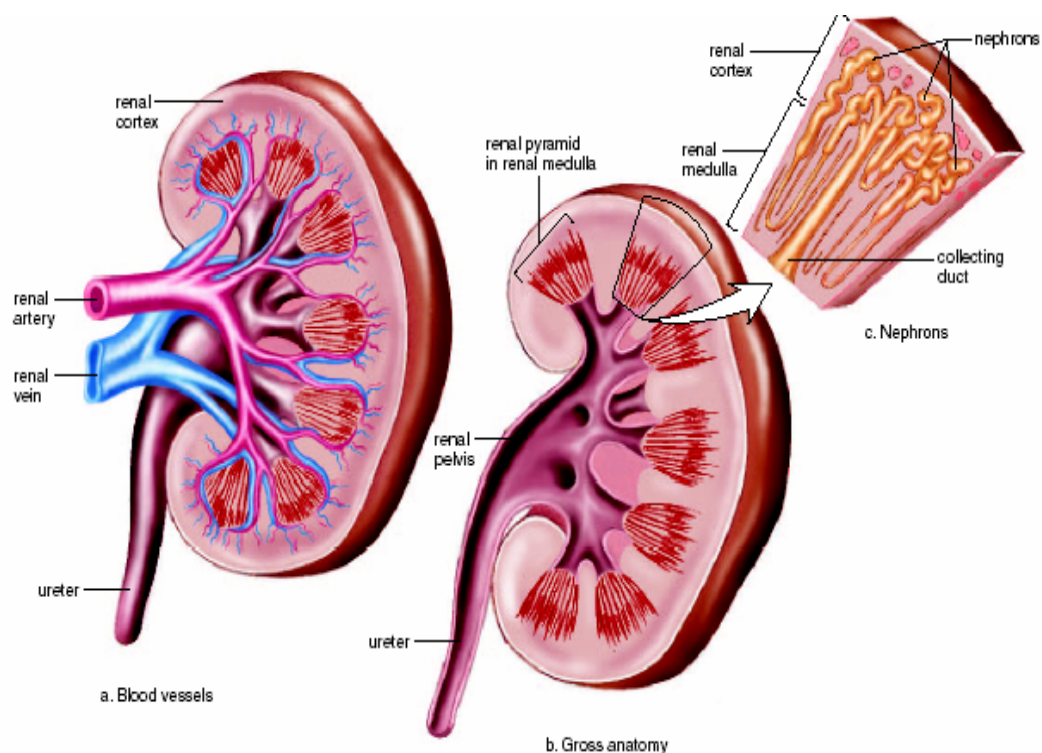


Figure 2-2. Gross anatomy of the kidney. **a.** A sagittal section of the kidney showing the blood supply. **b.** The same section showing the renal cortex, the renal medulla, and the renal pelvis, which connects with the ureter. **c.** An enlargement showing the placement of nephrons [81].

Each kidney contains over a million tiny structures called nephrons. Nephrons are the structural and functional units of the kidneys [80]. Each nephron is made up of several parts. First, the closed end of the nephron is pushed in on itself to form a cuplike structure called the glomerular capsule (Bowman's capsule). Next, there is a proximal convoluted tubule (PCT), then the tube narrows and makes a U-turn called the loop of the nephron (loop of Henle). Each loop consists of a descending limb and an ascending

limb. Then the distal convoluted tubule (DCT). The distal convoluted tubules of several nephrons enter one collecting duct. Many collecting ducts carry urine to the renal pelvis [81].

As shown in **Figure 2.2**, the glomerular capsule and the convoluted tubules always lie within the renal cortex. The loop of the nephron dips down into the renal medulla [81]. Each nephron is associated with two capillary beds- the glomerulus and the peritubular capillary bed [80]. From the renal artery, an afferent arteriole leads to the glomerulus, a knot of capillaries inside the glomerular capsule. Blood leaving the glomerulus enters the efferent arteriole. The efferent arteriole takes blood to the peritubular capillary network, which surrounds the rest of the nephron. From there, the blood goes into a venule that joins the renal vein **Figure 2.3** [81].

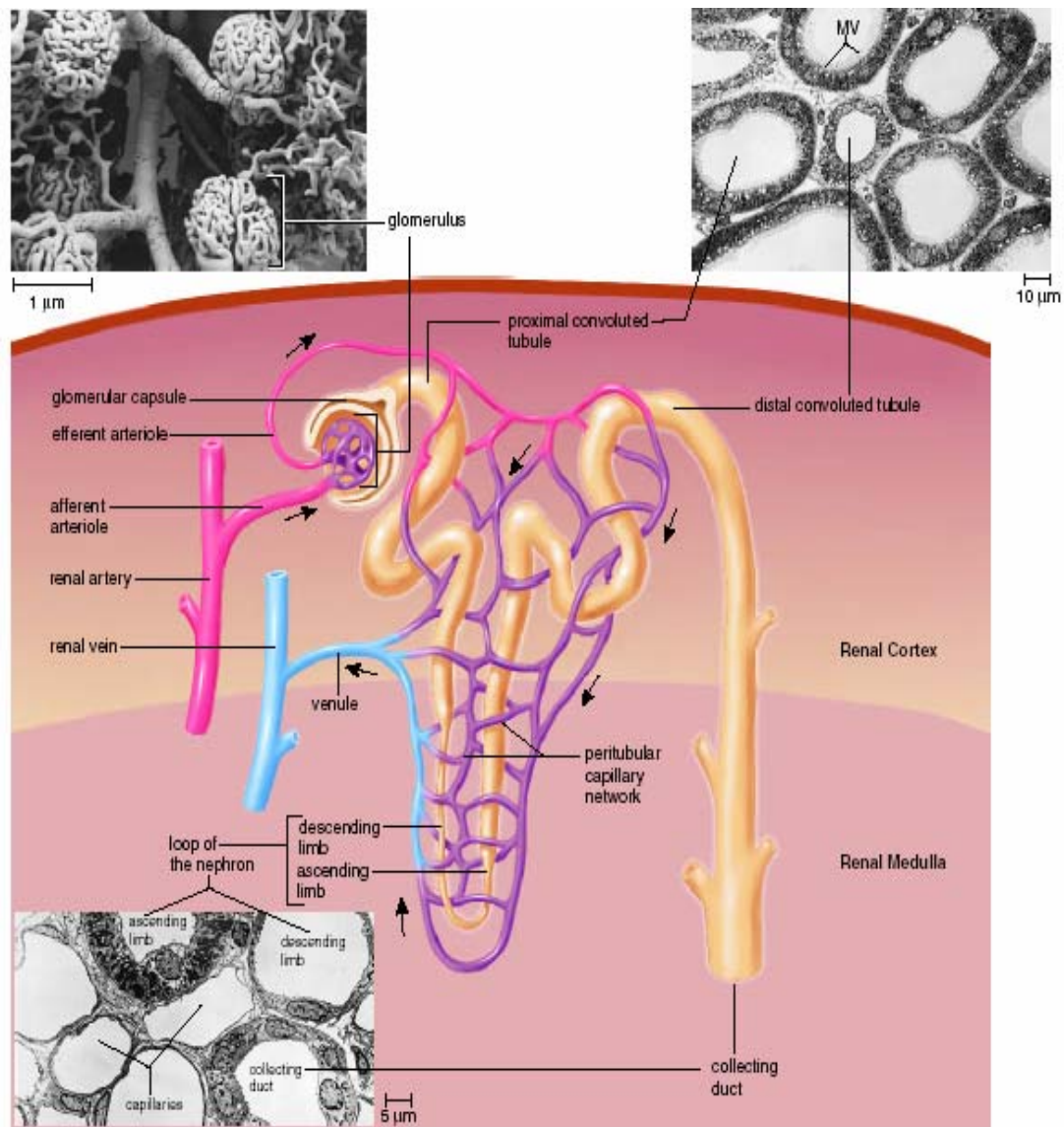


Figure 2.3. Nephron anatomy. A nephron is made up of a glomerular capsule, the proximal convoluted tubule, the loop of the nephron, the distal convoluted tubule, and the collecting duct [81].

2.4.1 Excretory function of the kidney

The three processes by which the kidneys adjust the composition of plasma are filtration, reabsorption, and secretion.

A – Filtration : the glomerulus acts as a filter. Filtration is a non selective, passive process. Water and solutes smaller than proteins are forced through the capillary walls and pores of the glomerular capsule into the renal tubule [80].

B – Reabsorption : tubular reabsorption begins as soon as the filtrate enters the proximal convoluted tubule. Many useful substances including water, glucose, amino acids, and needed ions, are transported out of the filtrate into the tubule cells and then enter the

capillary blood. Most reabsorption occurs in the proximal convoluted tubules but under certain conditions the distal convoluted tubule and the collecting duct are also active [80].

C- Secretion : tubular secretion is essentially reabsorption in reverse. Some substances, such as hydrogen and potassium ions and creatinine, move from the blood of the peritubular capillaries through the tubule cells or from the tubule cells themselves into the filtrate to be eliminated in urine [80].

2.4.2 Other functions of the kidney

- Regulate blood pressure, by producing the enzyme rennin.
- Stimulate red blood cell production in bone marrow, by producing erythropoietin hormone.
- Kidney cells also convert vitamin D to its active form, calcitriol [80].

2.4.3 Kidney disease

Renal disease may be acute or chronic. Acute renal failure is worsening of renal function over hours to days, resulting in the retention of nitrogenous wastes such as urea nitrogen and creatinine in the blood. Chronic renal failure results from an abnormal loss of renal function over months to years. Chronic renal disease is rarely reversible and leads to progressive decline in renal function. Reduction in renal mass leads to hypertrophy of the remaining nephrons with hyperfiltration, and the glomerular filtration rate in these nephrons are transiently at supranormal levels, that may worsen renal function [11].

2.4.4 Stages of CKD

K/DOQI published a classification of the stages of CKD, as follows:

- Stage 1: Kidney damage with normal or increased GFR (>90 mL/min/1.73 m²)
- Stage 2: Mild reduction in GFR (60-89 mL/min/1.73 m²)
- Stage 3: Moderate reduction in GFR (30-59 mL/min/1.73 m²)
- Stage 4: Severe reduction in GFR (15-29 mL/min/1.73 m²)
- Stage 5: Kidney failure (GFR <15 mL/min/1.73 m² or undergoing dialysis) [5].

2.5 Hemodialysis

In renal failure, filtrate formation decreases or stops completely. Because toxic wastes accumulate quickly in the blood when the kidney tubule cells are not working, dialysis by an artificial kidney is necessary to cleanse the blood while the kidneys are shut down [80].

The basic principle of the artificial kidney is to pass blood through minute blood channels bounded by a thin membrane. On the other side of the membrane is a dialyzing fluid into which unwanted substances in the blood pass by diffusion. The rate of movement of solute across the dialyzing membrane depends on (1) the concentration gradient of the solute between the two solutions, (2) the permeability of the membrane to the solute, (3) the surface area of the membrane and (4) the length of time that the blood and fluid remain in contact with the membrane. Thus the maximum rate of solute transfer occurs initially when the concentration gradient is greatest (when dialysis is begun) and slows down as the concentration gradient is dissipated [82].

The removal of free water during HD is called ultrafiltration. It occurs when water driven by either a hydrostatic or osmotic force is pushed through the membrane of the dialyzer [19].

For the majority of patients with chronic renal failure, between 9 and 12 h of dialysis is required each week, usually divided into three equal sessions. However, the dialysis dose must be individualized. Recently there has been much interest in the possibility that more frequent dialysis may be associated with improved outcomes in patients [83].

2.5.1 Epidemiology and etiology

The population of persons with ESRD is increasing in the United States. The prevalence of CKD in adults in the United States was 11% in 1988 through 1994 and 11.7% in 1999 through 2000 [84]. The prevalence of CKD in the United States in 1999-2004 is higher than it was in 1988-1994. This increase is partly explained by the increasing prevalence of diabetes and hypertension and raises concerns about future increased incidence of kidney failure and other complications of CKD [85]. Total CKD prevalence in Norway was 10.2% in 1995 through 1997. ESRD incidence is much lower in Europe compared with the United States [84].

In 2001, 1,479,000 people were undergoing treatment for ESRD worldwide. Of these, 1,015,000 underwent HD [86].

In the United States the ESRD incidence is 338 per million population (*versus* 90 [Finland] to 170 [Germany]) in 2003 [87].

The dialysis population is increasing worldwide with prevalence of 215 patients per million population on dialysis in 2004 [88].

In Jordan, the number of patients on HD has at least doubled over the past 5 years [89].

At present, there are around 2500 patients on maintenance HD therapy in Libya [90].

The number of ESRD patients on renal replacement therapy in Iran reached about 25000 in 2006. The prevalence and incidence of ESRD are 357 and 66 per million population, respectively [91].

The overall 5-year mortality among HD patients was 51.4% in Libya, 40% in USA, 44-48% in Saudi Arabia, and 48% in the UK [90].

Over 50% of cases of CKD are due to diabetes mellitus and hypertension.

Glomerulonephritis, cystic diseases, and other urologic diseases account for another 20-25%, and nearly one-sixth of patients have unknown causes. [11].

2.5.2 Criteria for placing patients on dialysis

Commonly accepted criteria for placing patients on dialysis include the presence of the uremic syndrome; the presence of hyperkalemia unresponsive to conservative measures; extracellular volume expansion; acidosis refractory to medical therapy; a bleeding diathesis; and a creatinine clearance of 10 mL/min per 1.73 m² [83].

2.5.3 Medical management of hemodialysis patients

The aim of management is to reduce the occurrence of uremic bone disease and cardiovascular morbidity and mortality caused by elevated serum levels of PTH and calcium × phosphorus product. Efforts to manage phosphorus retention in patients with ESRD almost always entail the use of phosphate-binding agents together with phosphorus restriction [92]. Daily dietary phosphorus intake is recommended to be limited to 800-1000 mg daily when the serum phosphorus level is > 5.5 mg/dl [93].

Phosphate binders

Calcium- based phosphate binders are often recommended as the initial binder therapy [94]. The total dosage of elemental calcium provided by the calcium- based phosphate binders should not exceed 1500 mg/day, and the total intake of elemental calcium (including dietary calcium) should not exceed 2000 mg/day [10]. Extensive clinical experience is available for sevelamer hydrochloride which has proved to be equivalent, although not superior, to calcium carbonate in terms of its phosphate chelating capacity [95]. Lanthanum carbonate is an alternative noncalcium phosphate binder [96].

In patients undergoing dialysis and who remain hyperphosphatemic despite the use of calcium-based phosphate binders or other noncalcium phosphate binders, a combination of both has been recommended . In such patients, more frequent dialysis should also be considered [97].

Medical management of secondary hyperparathyroidism

In stage 5, CKD, therapy with an active vitamin D sterols (calcitriol, alfacalcidol, paricalcitol or doxercalciferol) should be provided if the plasma concentration of PTH is above 300 pg/ml [98]. Intermittent, intravenous administration (known as puls dosing) of calcitriol is more effective than daily oral calcitriol in lowering plasma PTH levels [99]. Calcimimetic agents are small organic molecules that activate the CaR in the membrane of the parathyroid cell, thereby inhibiting PTH release. They represent a novel approach to managing excess PTH secretion because their mechanism of action is distinct from that of the vitamin D sterols, and their efficacy in lowering plasma PTH concentrations in hemodialysis patients with secondary hyperparathyroidism has been documented in several clinical trials [100]. Calcimimetic agents retard the development of parathyroid gland hyperplasia [101]. Cinacalcet HCl is a new Calcimimetic agent that is currently approved for clinical use [79].

2.6 Related studies

In a study performed on 57 HD patients, who had been on dialysis for more than 9 months. The percentage of patients whose serum Ca, P, Ca x P product and PTH were within K/DOQI recommended target ranges were 46%, 53%, 77% and 28%, respectively [2].

In another study performed on 140 patients, in USA, over a 6-month period in an inner city HD unit, it was found that the level of serum calcium and serum phosphorus fell within the range recommended by the (K/DOQI) guidelines 49% and 36% of the time respectively, 57% of the determination for Ca x P product were < 55 mg²/dl². PTH levels were within the recommended values in 20% of the determinations. Only 7% of the determinations met all four criteria simultaneously [102].

In another study, performed on Seventy-three patients, in Turkey, (46 male, 27 female; mean age 48±13 years, on HD for 82±80 months), the percentage of patients who met all three targets of NKF-K/DOQI for phosphate, calcium and Ca x PO₄ product was 27.1%, whereas those who did not achieve the target in one, two or three parameters was 28.1%, 17.7% and 14.6%, respectively [103].

In another study, the state of mineral metabolism (serum PTH, phosphorus, calcium, and calcium-phosphorus product) was described for representative samples of patients and facilities from 7 countries (France, Germany, Italy, Japan, Spain, United Kingdom, and United States) participating in the Dialysis Outcomes and Practice Patterns Study (DOPPS I, 1996-2001; DOPPS II, 2002-2004). Results: A relatively modest percentage of patients fell within the guideline range for PTH (21.4% in DOPPS I, 26.2% in DOPPS II), serum phosphorus (40.8%, 44.4%), albumin-corrected serum calcium (40.5%, 42.5%), and calcium-phosphorus product (56.6%, 61.4%). Results were not dramatically different across countries. The majority of patients not within guideline ranges had high serum levels of phosphorus (51.6% in DOPPS I, 46.7% in DOPPS II), calcium (50.1%, 48.6%), and calcium-phosphorus product (43.4%, 38.6%) and low (<150 pg/mL) concentrations of PTH (52.9%, 47.5%). It was rare for patients to fall within recommended ranges for all indicators of mineral metabolism; 23% to

28% fell within guideline for at least 3 measures and only 4.6% to 5.5% of patients were within range for all 4 [104].

A multicentre observational study (COSMOS), performed on randomly selected hemodialysis patients, (1469 men, 1026 women), recruited from 285 facilities in 21 countries. The investigators assessed outcomes in relation to the percentage of patients who fell within KDOQI target ranges for serum calcium, phosphorous, calcium–phosphorus multiplication product and parathormone. That goal was achieved by 53.5%, 52.2%, 75.6%, and 32.4% of the participants, respectively [105].

Chapter 3

Materials and Methods

3.1. Study design

The present study is a case-control study.

3.2. Target population

The target population is hemodialysis patients diagnosed as ESRD on hemodialysis for ≥ 12 months. All patients were on phosphate binder (calcium carbonate), while 55 patients were receiving vitamin D analogue (0.5 μg alfacalcidol daily), and the other 25 patients were not.

3.3. Setting of the study

The hemodialysis unit at Al-Shifa hospital in Gaza strip.

3.4. Sample size

The present study included 160 subjects divided as 80 cases and 80 healthy individuals as control sample. Controls and cases were age and sex matched.

3.5. Ethical considerations

The necessary approval to conduct the study from Helsinki Committee in the Gaza strip was obtained. This approval was issued on March 2008(Annex 1). Helsinki Committee is an authorized professional body for giving permission to researchers to conduct their studies with ethical concern in the area. Two official letters of request were sent to both the director of Al-Shifa hospital (Annex 2) and the director of Beet-Hanoun hospital (Annex 3) for approval to carry out the study in the hospitals and laboratories. The participants were given a full explanation about the purpose of the study and assurance about the confidentiality of the information obtained through the questionnaire and blood analysis.

3.6. Data collection

An interview used for filling in the questionnaire which was designed for matching the study need. Face to face interviews were conducted by the researcher to

collect data from both the patients (Annex 4) and controls (Annex 5). The questionnaire included personal and medical information.

3.7. Determination of BMI

BMI (kg/m^2) was determined by dividing weight (in kilograms) by height (in squared meters).

3.8. Blood sampling and processing

Blood samples were collected by the researcher from all the subjects who agreed to participate in the study (before HD sessions for the patients). Five ml blood were obtained from each subject into vacutainer plain tubes and were left short time to allow blood to clot, then serum samples were obtained by centrifugation at 3000 rpm for 15 minutes.

The serum of the patients samples were separated in the laboratory of Al- Shifa hospital, while the blood samples of the controls were collected and centrifuged in the laboratory of Beet- Hanoun hospital. Each subject's serum was divided into two tubes and kept at -70°C in the laboratories of the Islamic University until the time of performing the analysis.

Serum urea, creatinine, albumin, total calcium, ionized calcium, and phosphorus analyses were carried out in the laboratory of Beet- Hanoun hospital, while PTH assay was carried out in a private licensed laboratory (Balsam laboratory).

3.9. Biochemical analysis

Serum urea, creatinine, albumin, total calcium, and phosphorus were analyzed manually using Biosystem BTS-310 spectrophotometer (Spain). Two levels of lyophilized multi-control sera ; normal and abnormal levels were analyzed with each run. Serum ionized calcium was analyzed using ISE method by AVL 9180 Electrolyte analyzer, Roche, Germany. Three levels of controls; I, II, III, were analyzed with each run. PTH was analyzed using ELISA method by ELISA reader, Diamed, (Italy). Low and high level of the DSL controls were analyzed with each run.

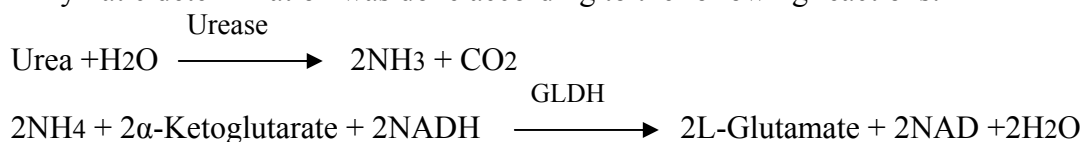
3.9.1. Determination of serum urea

Serum urea was determined using AMP Diagnostics kit

Method : Enzymatic U. V.

Principle

Enzymatic determination was done according to the following reactions:



GLDH = Glutamate dehydrogenase

Reagents composition

Reagent : R1

Tris buffer, pH 7.60	125	mmol/L
ADP	1	mmol/L
α -Ketoglutarate	9	mmol/L
Urease	≥ 8100	U/L
GLDH	≥ 1350	U/L

Reagent : R2

NADH	1.5	mmol/L
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Preparation and stability of working reagent

Four volumes of R1 were mixed with 1 volume of R2

Stability : 5 days at 20-25° C
4 weeks at 2-8° C

Procedure

Wavelength	:	340 nm (334-365)
Temperature	:	37° C
Cuvette	:	1 cm light path

Reading against distilled water was performed.

one reagent procedure

Working reagent	:	1 ml
Sample or standard	:	10 μ l

Mixing and reading the variation of optical density (ΔOD) between 30 seconds and 90 seconds was performed.

Calculation

$$\frac{\Delta OD \text{ Sample}}{\Delta OD \text{ Standard}} \times n = \text{urea sample concentration (mg/dl)}$$

n = standard urea concentration (50 mg/dl)

3.9.2. Determination of serum creatinine

Serum creatinine was determined according to Jaffe method using AMP Diagnostics kit

Method : Kinetic

Principle

The rate of formation of a coloured complex between creatinine and alkaline picrate is measured. The effect of interfering substances are reduced using the kinetic procedure.

Reagents composition

Reagent : R1

Picric acid	8.73	mmol/L
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Reagent : R2

Sodium hydroxide	312.5	mmol/L
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Disodium phosphate	12.5	mmol/L
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Standard : Std

Creatinine	2	mg/dl
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Preparation and stability of working reagent

Mixing 1 volume of R1 with 1 volume of R2 was performed.

Stability : 1 month at 20-25 ° C

Procedure

Wavelength : 492 nm (480-520)

Temperature : 37° C

Cuvette : 1 cm light path

Reading against distilled water was performed.

Working reagent	:	1 ml
Sample or standard	:	100 µl

Mixing and reading the optical density (OD1) 10 seconds after the sample or standard addition was performed. Exactly 2 minutes after the first reading, the second reading (OD2) was done.

Calculation

$$\frac{\Delta OD \text{ Sample}}{\Delta OD \text{ Standard}} \times n = \text{sample creatinine concentration (mg/dl)}$$

n = standard creatinine concentration

3.9.3. Determination of serum albumin

Serum albumin was determined using AMP Diagnostics kit

Method : Colorimetric.
Bromocresol green (BCG).

Principle

Colorimetric determination of serum albumin using Bromocresol green (BCG) at pH 4.20.

Reagents composition

Reagent : R			
	Succinate buffer, pH 4.20	87	mmol/L
	Bromocresol green	0.2	mmol/L
	Brij 35	7.35	ml/L
Standard : Std	Bovine albumin	5	g/dL

Preparation and stability of working reagent

The reagent is ready for use

Procedure

Wavelength : 628 nm
Temperature : 37° C
Cuvette : 1 cm light path

Reading against reagent blank was performed.

	BLANK	STANDARD	SAMPLE
R	1 mL	1 mL	1 mL
Distilled water	10 µL	-	-
Std	-	10 µL	-
Sample	-	-	10 µL

Mixing and reading the optical density (OD) after a 5 minute incubation was done.

The final color is stable for at least 15 minutes.

Calculation

$$\frac{\text{OD Sample}}{\text{OD Standard}} \times n = \text{sample albumin concentration (gm/dL)}$$

n = standard albumin concentration

3.9.4. Determination of serum calcium

Serum calcium was determined using Diagnostic Systems International kit

Method :

Photometric test using cresolphthalein complexone (CPC)

Principle

Cresolphthalein complexone reacts with calcium ions in alkaline medium forming a red-violet color. Interference by magnesium is eliminated by addition of 8-hydroxy-quinoline.

Reagents composition

Reagent : R1

Ethanolamine Detergent pH 10.7 600 mmol/L

Reagent : R2

2- Cresolphthalein complexone	0.06	mmol/L
8-hydroxyquinoline	7	mmol/L
Hydrochloric acid	20	mmol/L

Standard : Std	10	mg/dl
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Preparation and stability of working reagent

Four parts of R1 were mixed with 1 part of R2

Stability : 3 days at 2–8 °C

Procedure

Wavelength : 570 nm, Hg 578 nm (550 – 590 nm)
 Temperature : 37° C
 Cuvette : 1 cm light path

Reading against reagent blank was done.

	BLANK	STANDARD	SAMPLE
Working reagent	1 mL	1 mL	1mL
Distilled water	20 µL	-	-
Std	-	20 µL	-
Sample	-	-	20 µL

Mixing and reading the optical density (OD) after a 5 minute incubation was done. The final color is stable for at least 15 minutes.

Calculation

$$\frac{\text{OD Sample}}{\text{OD Standard}} \times n = \text{sample calcium concentration (mg/dL)}$$

n = standard calcium concentration

Note :

Albumin-corrected serum calcium (mg/dL) = measured total calcium + 0.8 [4.0- serum albumin (g/dL)] [106]

3.9.5. Determination of serum phosphorus

Serum phosphorus was determined using AMP Diagnostics kit

Method

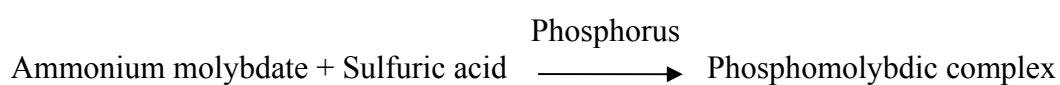
Phosphomolybdate.

U.V.

End point

Principle

Determination of inorganic phosphorus is made according to the following reaction:



Reagents composition

Reagent : R

Sulfuric acid	210	mmol/L
Ammonium molybdate	650	umol/L

Standard : Std

Phosphorus	5	mg/dl
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Preparation and stability of working reagent

The reagent is ready for use.

Procedure

Wavelength	:	340 nm
Temperature	:	37° C
Cuvette	:	1 cm light path

Reading against reagent blank was done.

	BLANK	STANDARD	SAMPLE
Reagent R	1 mL	1mL	1mL
Distilled water	10 uL	-	-
Std	-	10uL	-
Sample	-	-	10uL

Mixing and reading the optical density (OD) after a 5 minute incubation was done.
The final color is stable for at least 1 hour.

Calculation

$$\frac{\text{OD Sample}}{\text{OD Standard}} \times n = \text{sample phosphorus concentration (mg/dL)}$$

n = standard phosphorus concentration

Note :

(Calcium \times Phosphorus) product (mg^2/dL^2) was obtained by multiplying the result of albumin-corrected serum calcium (mg/dL) with the result of serum phosphorus (mg/dL)

3.9.6. Determination of serum parathormone

Serum PTH was determined using the Diagnostic Systems Laboratories, Inc. Corporate Headquarters, 445 Medical Center Blvd. Webster, Texas 77598-4217 USA Kit.

Intended use

The DSL-10-8000 Active® I-PTH Enzyme-Linked Immunosorbent Assay (ELISA) Kit provides materials for the quantitative measurement of I-PTH in serum or plasma.

Principle of the test

The DSL-10-8000 Active® I-PTH ELISA is an enzymatically amplified two-step sandwich-type immunoassay. In the assay, standards, controls, and unknowns were incubated with biotinylated anti-parathormone antibody of defined specificity in micro-titration wells precoated with an affinity purified goat anti- human PTH antibody of defined and unique epitope specificity. After incubation and washing, the wells were treated with streptavidin labeled with enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells were incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution was then added and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm. The absorbance measured is directly proportional to the concentration of I-PTH present. A set of I-PTH standards was used

to plot a standard curve of absorbance versus I-PTH concentration from which the I-PTH concentration in the unknowns can be calculated.

Reagents

- Anti-I-PTH- coated microtitration strips : one stripholder containing 96 microtitration wells coated with anti I-PTH affinity-purified antibody
- I-PTH standard A/sample diluent : one vial, 5 ml, labeled A, containing 0 pg/ml I-PTH in a buffered matrix.
- I-PTH standards B–G : six vials, 1 ml each, labeled B-G, containing concentrations of approximately 10.0, 30.0, 100.0, 250.0, 750.0 and 2000.0 pg/ml I-PTH in a buffered human serum-based matrix .
- I-PTH controls : two vials, levels I and II, containing low and high concentrations of I-PTH.
- I-PTH assay buffer : one vial, 11 ml, containing a phosphate buffered matrix.
- I-PTH antibody-biotin conjugate : one vial, 11 ml, containing anti- I-PTH antibody conjugated to biotin in protein-based buffer.
- Streptavidin-enzyme conjugate concentrate : one vial, 0.3 ml containing Streptavidin-enzyme conjugate.
- TMB chromogen solution : one bottle, 11 ml, containing a solution of tetramethylbenzidine (TMB) in citrate buffer with hydrogen peroxide.
- Wash concentrate : one bottle, 100 ml, containing buffered saline with a nonionic detergent.
- Stopping solution : one vial, 11 ml, containing 0.2M sulfuric acid.

Reagent preparation:

- Wash solution : by diluting the wash concentrate 10-fold with deionized water.
- Streptavidin-enzyme conjugate solution : by diluting the Streptavidin-enzyme conjugate concentrate at a ratio of 1 part into 50 parts of the I-PTH assay buffer.

Assay procedure

Note : All reagents and samples were allowed to reach room temperature (~25°C) and mixed thoroughly by gentle inversion before use.

- 1- The microtitration strips to be used, were marked.

- 2- Fifty μL of the standards, controls, and unknowns were pipetted to the appropriate wells.
- 3- Then, 100 μL of the antibody-biotin conjugate solution was added to each well .
- 4- Incubation of the wells, shaking at 500-700 rpm on an orbital microplate shaker, for 2.5 hours at room temperature ($\sim 25^{\circ}\text{C}$) was performed.
- 5- Aspiration and washing each well five times with the wash solution using an automatic microplate washer was performed. Blot dry by inverting the plate on absorbent material was done.
- 6- Then, 100 μL Streptavidin-enzyme conjugate solution was added to each well.
- 7- Incubation of the wells, shaking at 500-700 rpm on an orbital microplate shaker, for 30 minutes at room temperature ($\sim 25^{\circ}\text{C}$) was performed.
- 8- Aspiration and washing each well five times with the wash solution using an automatic microplate washer was performed. Blot dry by inverting the plate on absorbent material was done.
- 9- Then, 100 μL of the TMB solution was added to each well .
- 10- Incubation of the wells, shaking at 500-700 rpm on an orbital microplate shaker, for 10-15 minutes at room temperature ($\sim 25^{\circ}\text{C}$) was performed. Exposure to direct sunlight was avoided.
- 11- Then, 100 μL of the stopping solution was added to each well.
- 12- Reading the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm was done.

Calculation

- A. The mean absorbance for each standard, control or unknown was calculated.
- B. The log of the mean absorbance readings for each of the standards along the y-axis versus the log of the I-PTH concentrations in pg/ml along the x- axis, using a linear curve-fit, was plotted.
- C. The I-PTH concentrations of the controls and unknowns were determined from the standard curve by matching their mean absorbance readings with the corresponding I-PTH concentrations.

Expected values

The following data may be used as a guideline as published by the manufacturer.

Subject	Absolute range (pg/mL)	Mean \pm SD (pg/mL)
Normal Adults	16 - 62	33 \pm 12
Primary Hyperparathyroidism	50 - 962	341 \pm 357
Renal failure with secondary hyperparathyroidism	50 - 1238	491 \pm 340

3.9.7. Determination of serum free- ionized calcium

Serum free- ionized calcium was determined using ion-selective electrode method. The instrument used was AVL 9180 electrolyte analyzer (Germany) which perform self calibration and when the calibration process is correctly performed, the screen of the instrument display ready status and the serum ionized calcium can be measured. Three levels of controls; I, II, III, were analyzed with each run.

3.10. Statistical analysis

Data were computer analyzed using SPSS (Statistical Package for Social Science), version 11 with the following steps :

- 1- Data entry
- 2- Data cleaning
- 3- Data tabulation
- 4- Data cross-tabulation

Then the variables of the study were conducted to multiple statistical tests according to types of variables, such as (t- test, chi-square test (X^2), and correlation and regression test). Results analyzed were expressed as mean \pm SD.

The result was statistically significant when the P-value was less than 0.05.

Chapter 4

Results

4.1. Study population description

The sample size of the study was 160 subjects. The case-control ratio was 1:1 matched by age and gender. The percentage of males was 51%, while the percentage of females was 49% (**Figure 4.1**). The mean age was 47.2 ± 15.9 years among cases and 47.3 ± 15.8 years among controls. The percentage of the subjects from Gaza governorate was 55% (66.2% of cases, 43.7% of controls), while those from North governorates was 45% (33.8% of cases, 56.3% of controls) (**Figure 4.2**)

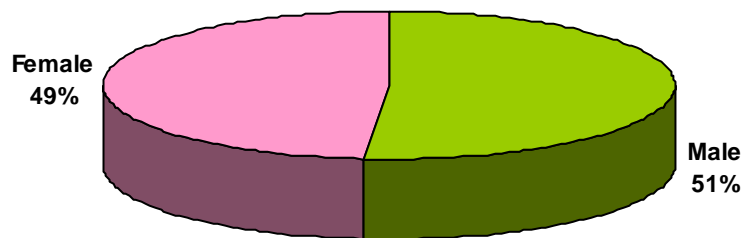


Figure 4.1. Distribution of study population by gender

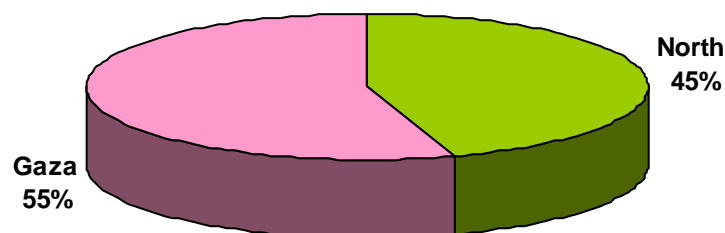


Figure 4.2. Distribution of study population by Governorates

The average of weight was 69.9 ± 20.8 kg among cases and 70.7 ± 10.7 kg among controls (**Figure 4.3**), The average of height was 1.6 ± 0.1 m among cases and 1.7 ± 0.1 m among controls) (**Figure 4.4**). The average of BMI was 25.5 ± 6.7 kg/m² among cases, 23.3 ± 2.5 kg/m² among controls (**Figure 4.5**).

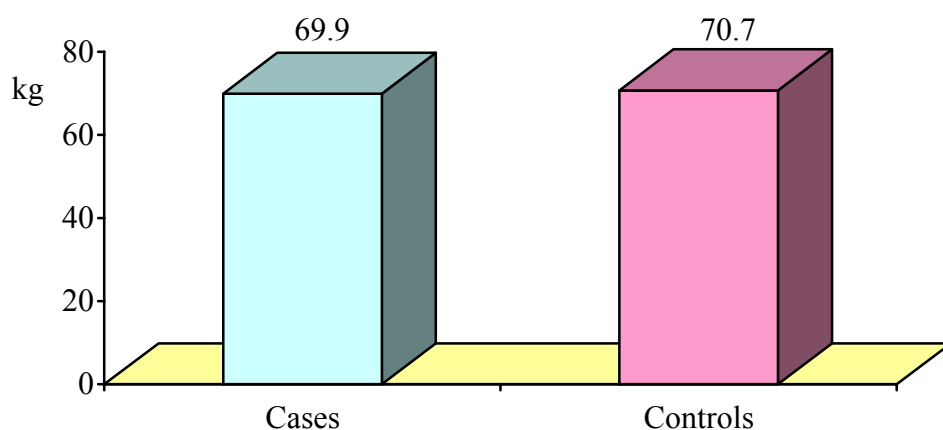


Figure 4.3 Average of weight among cases and controls

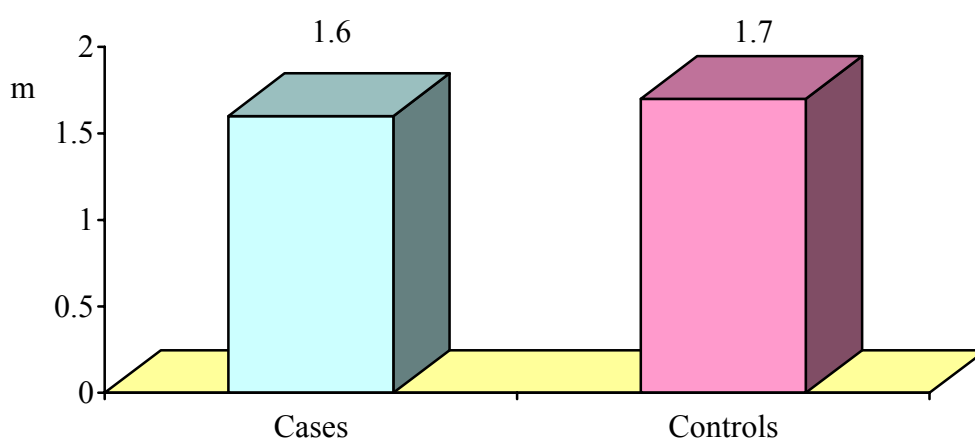


Figure 4.4 Average of height among cases and controls

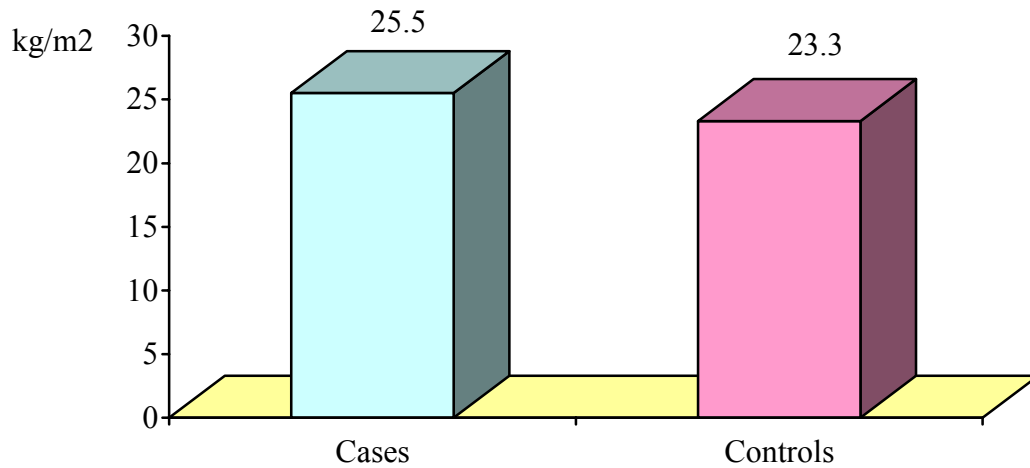


Figure 4.5. Averages of BMI among cases and controls

The mean of frequency of hemodialysis among cases was 2.6 ± 0.5 sessions weekly. The mean of duration of hemodialysis among cases was 49.1 ± 38.8 months (**Figure 4.6**). Also, the percentage of cases on hemodialysis for less than 48 months was 49 (61.3%), while those on hemodialysis for 48 months or more was 31 (38.7 %) (**Figure 4.7**). The percentage of cases on hemodialysis 2 sessions weekly was 31 (38.7%) while those on hemodialysis 3 sessions weekly was 49 (61.3%) (**Figure 4.8**). The percentage of cardiovascular diseases was 22.5% among cases, none among controls, and the percentage of bone disorders was 46.3 % among cases, none among controls (**Figure 4.9**).

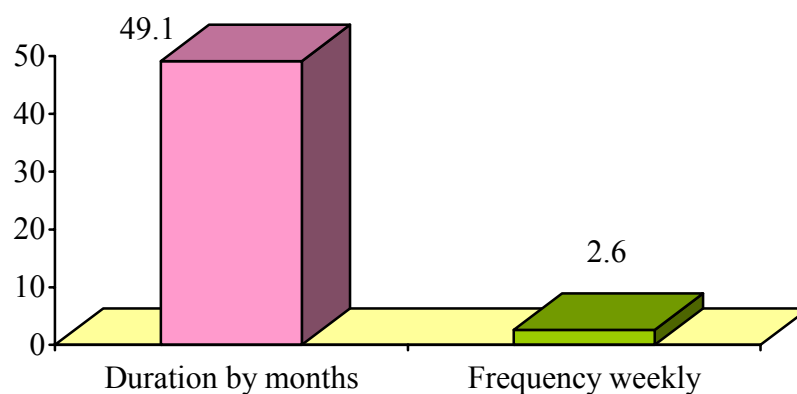


Figure 4.6. Averages of duration and frequency of hemodialysis among cases

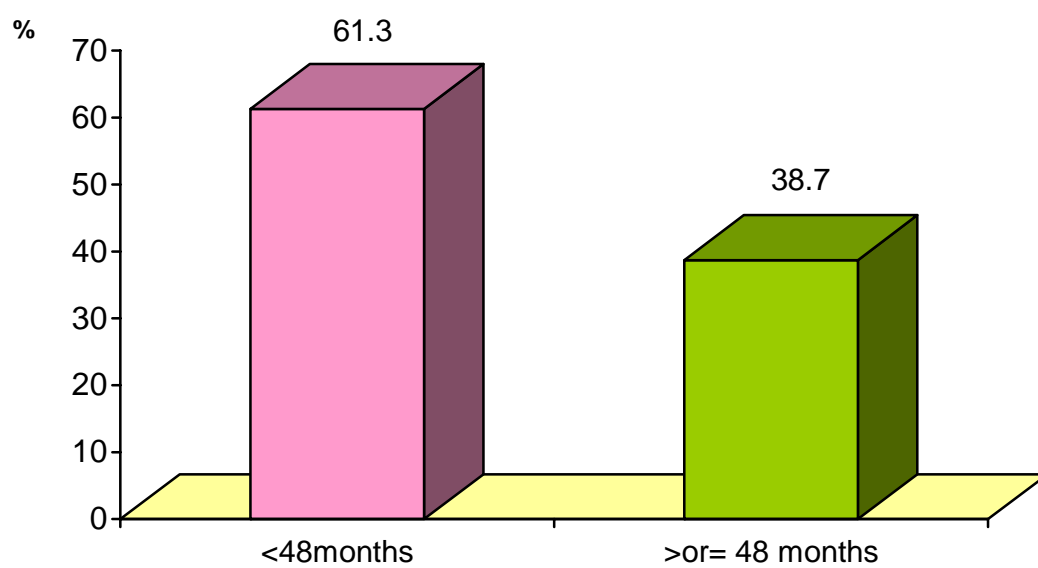


Figure 4.7. Distribution of cases by duration of hemodialysis

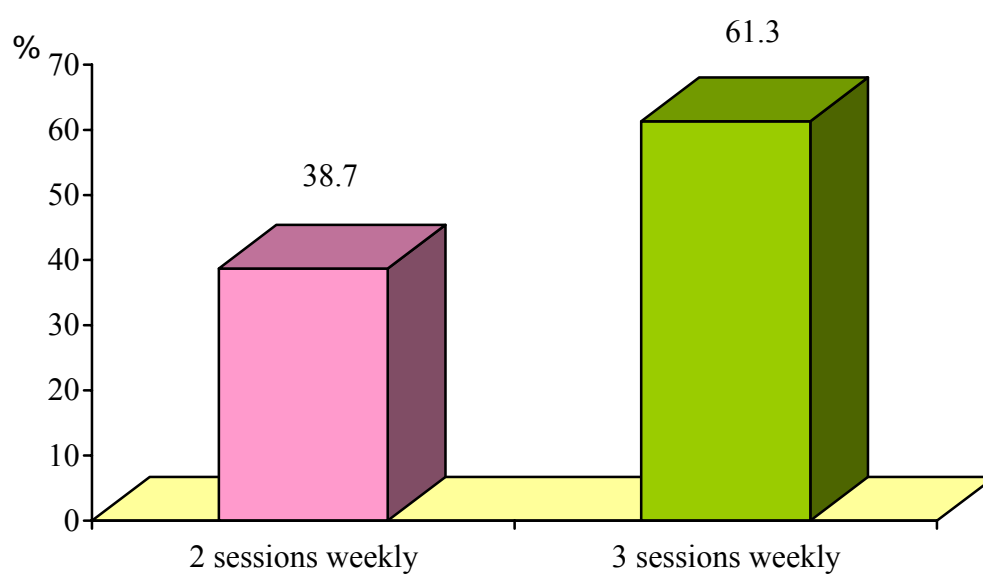


Figure 4.8. Distribution of cases by frequency of hemodialysis

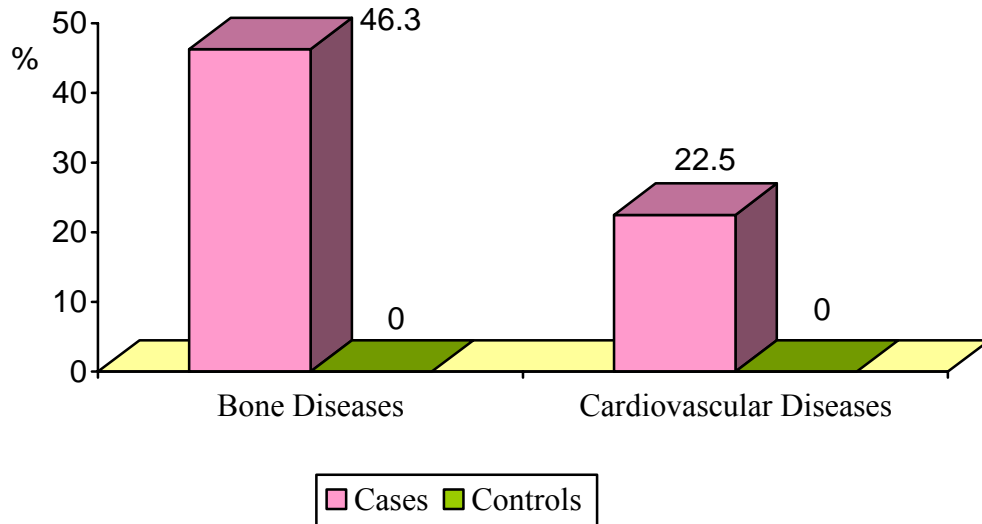


Figure 4.9. Distribution of cases and controls by bone and cardiovascular diseases

4.2. Relationship between parathormone and calcium× phosphorus product, albumin- corrected serum calcium and serum phosphorus with gender among cases and controls

Table 4.1 reveals that there was no significant difference ($t=-0.20$, $p=0.840$) between male cases (1677.4 ± 1807 pg/ml) and female cases (1755 ± 1616.2 pg/ml) regarding parathormone average. Also, there was no significant difference ($t=-1.93$, $p=0.057$) between male cases (59.70 ± 12.4 mg²/dl²) and female cases (56.9 ± 16.1 mg²/dl²) for (Ca × P) product average. The same results was observed with albumin-corrected serum Ca, ($t=-1.5$, $p=0.129$), and with serum phosphorus ($t=-1.3$, $p=0.185$) among male cases (9.4 ± 1.0 mg/dl, 6.4 ± 1.2 mg/dl respectively) and female cases (9.7 ± 0.8 mg/dl, 6.8 ± 1.6 mg/dl respectively).

Table 4.1. Averages of parathormone, (Ca × P) product, albumin - corrected serum Ca, and serum phosphorus among cases by gender

Variable	Male (n=41)	Female (n=39)	P-value
	Mean \pm SD	Mean \pm SD	
PTH (pg/ml)	1677.4 \pm 1807	1755 \pm 1616.2	0.84
Ca \times P (mg ² /dl ²)	59.70 \pm 12.4	56.9 \pm 16.1	0.057
Albumin - corrected serum Ca (mg/dl)	9.4 \pm 1.0	9.7 \pm 0.8	0.129
Serum phosphorus (mg/dl)	6.4 \pm 1.2	6.8 \pm 1.6	0.185

P < 0.05: significant

Table 4.2 reveals that there was no significant difference (t=0.06, p=0.954) between male controls(35.8 \pm 16.8 pg/ml) and female controls (35.6 \pm 12.4 pg/ml) according to parathormone average. Also the table revealed that there was no .significant difference (t=0.9, p=0.395) between male controls (40.7 \pm 7.3 mg²/dl²) and female controls (39.6 \pm 4.3 mg²/dl²) by (Ca \times P) product average. The same results was observed according to albumin- corrected serum Ca (t=-0.2, p=0.821) and serum phosphorus (t=0.9, p=0.361) among male controls (9.4 \pm 0.3 mg/dl, 4.3 \pm 0.7 mg/dl respectively) and female controls(9.4 \pm 0.3 mg/dl, 4.2 \pm 0.4 mg/dl respectively).

Table 4.2. Averages of parathormone, (Ca × P) product, albumin - corrected serum Ca, and serum phosphorus among controls by gender

Variable	Male (n=41)	Female (n=39)	P-value
	Mean \pm SD	Mean \pm SD	
PTH (pg/ml)	35.8 \pm 16.8	35.6 \pm 12.4	0.954
Ca \times P (mg ² /dl ²)	40.7 \pm 7.3	39.6 \pm 4.3	0.395
Albumin - corrected serum Ca (mg/dl)	9.4 \pm 0.3	9.4 \pm 0.3	0.821
Serum phosphorus (mg/dl)	4.3 \pm 0.7	4.2 \pm 0.4	0.361

P < 0.05: significant

4.3. Levels of parathormone, calcium× phosphorus product, albumin-corrected serum calcium, and serum phosphorus among cases and controls:

As shown in **Table 4.3**, among the cases, the percentage of subjects whose PTH level is out of the range recommended by KDOQI guidelines was 86.2% (83.7 more than 300 pg/ml, 2.5% less than 150 pg/ml). In addition, about more than two third of cases (67.5%) have calcium × phosphorus product of 55 or more. Moreover, that more than half of cases (46.3% more than 9.5 mg/dl, 12.4% less than 8.4 mg/dl) have calcium level out of range recommended by KDOQI guidelines. Furthermore, more than three quarter of cases (77.5%) have phosphorus level more than 5.5 mg/dl.

Table 4.3. Distribution of cases by levels of parathormone, (Ca × P) product, albumin - corrected serum calcium and phosphorus

Variable	Groups	No	%
PTH level (pg/ml)	Less than 150	2	2.5
	150-300	11	13.8
	More than 300	67	83.7
(Ca × P) product (mg ² /dl ²)	Less than 55	26	32.5
	55 or more	54	67.5
Albumin - corrected serum Ca (mg/dl)	Less than 8.4	10	12.4
	8.4- 9.5	33	42.3
	More than 9.5	37	46.3
Serum phosphorus (mg/dl)	Less than 3.5	0	0
	3.5-5.5	18	22.5
	More than 5.5	62	77.5

As shown in **Table 4.4**, there was high statistically significant difference between the mean of parathormone level of cases (1715.3 ± 1706.3 pg/ml), range: 40-8778 pg/ml and the mean of parathormone level of controls (35.7 ± 14.7 pg/ml), range: 16-108 pg/ml ($t=8.80$, $p=0.000$). The mean of ($\text{Ca} \times \text{P}$) product among cases (62.7 ± 14.6 mg²/dl²), range: 31.2-105 mg²/dl², was significantly higher than that of controls (40.2 ± 6.0 mg²/dl²), range: 26 - 52.2 mg²/dl² ($t=12.79$, $p = 0.000$). Also, the difference between the mean of serum albumin of cases (4.6 ± 0.39 g/dl), range: 3.7 – 5.2 g/dl and the mean of serum albumin of controls (4.7 ± 0.3 g/dl), range: 3.8-5.2 g/dl was statistically significant ($t = -12.28$, $p = 0.024$).

The difference between the mean of albumin- corrected serum calcium of cases (9.5 ± 0.9 mg/dl), range: 7.3 – 11.0 mg/dl and that of controls (9.4 ± 0.3 mg/dl), range: 8.6 - 10.5 mg/dl was not statistically significant, ($t=0.86$, $p= 0.39$). On the other hand, the difference between the mean of free- ionized calcium of cases (3.78 ± 0.47), range: 2.9 – 4.9 mg/dl and that of controls (4.7 ± 0.1 mg/dl) range: 4.1 – 5.0 mg/dl, was statistically significant ($t=-17.33$, $p=0.000$). Also, the difference between the mean of serum phosphorus of cases (6.6 ± 1.4 mg/dl), range: 3.5 -10.5mg/dl and that of controls (4.3 ± 0.60 mg/dl), range: 2.8 -5.4 mg/dl, was statistically significant ($t=13.62$, $p=0.000$).

Table 4.4. Averages of parathormone, Ca×P product, serum albumin, albumin-corrected serum calcium , serum free- ionized calcium and serum phosphorus among cases and controls

Variable	Cases (n=80)	Controls (n=80)	P-value
	Mean \pm SD	Mean \pm SD	
PTH (pg/ml)	1715.3 ± 1706.3	35.7 ± 14.7	0.000*
Ca \times P (mg ² /dl ²)	62.7 ± 14.6	40.2 ± 6.0	0.000*
Serum albumin (g/dl)	4.60 ± 0.39	4.7 ± 0.3	0.024*
Albumin - corrected serum Ca (mg/dl)	9.50 ± 0.90	9.4 ± 0.3	0.394
Serum free- ionized Ca (mg/dl)	3.78 ± 0.47	4.7 ± 0.1	0.000*
P(mg/dl)	6.6 ± 1.4	4.3 ± 0.6	0.000*

*Statistically significant

Table 4.5, shows that the average of PTH, (Ca × P) product, and P among cases on hemodialysis for less than 48 months or those on hemodialysis for 48 months or more are higher than the values described by KDOQI guidelines. The averages of those parameters among cases on hemodialysis for 48 months or more was higher than parameters of those on hemodialysis for less than 48 months. It is important to mention that the difference in average of PTH level among cases on hemodialysis for less than 48 months (1401.4± 1485.2 pg/ml) and those on hemodialysis for 48 months or more (2211.3± 1929.3 pg/ml) was statistically significant (t=-2.11 , P= 0.038), while the other differences in the averages between the two groups of cases according to duration of hemodialysis were not significant statistically. Moreover, it is important to clarify that the averages of albumin- corrected serum Ca of both groups of cases according to duration of hemodialysis were close to the value recommended by KDOQI guidelines.

Table 4.5. Averages of parathormone, (Ca × P) product, albumin - corrected serum calcium and serum phosphorus among cases by duration of hemodialysis

Variable	Hemodialysis duration		P-value
	Less than 48 months (n=49)	48 months or more (n=31)	
	Mean ± SD	Mean ± SD	
PTH (pg/ml)	1401.4 ± 1485.2	2211.3 ± 1929.3	0.038*
Ca × P (mg ² /dl ²)	62.1 ± 15.4	63.7 ± 13.4	0.630
Albumin - corrected serum Ca (mg/dl)	9.5 ± 0.9	9.4 ± 0.9	0.311
P(mg/dl)	6.5 ± 1.5	6.8 ± 1.3	0.337

*Statistically significant

Concerning the frequency of hemodialysis, it was found that there were no statistically significant differences between the averages of PTH, (Ca × P) product, albumin- corrected serum Ca and serum P of cases on hemodialysis 2 sessions weekly and of those on hemodialysis 3 sessions weekly. However, it was observed that the average of PTH among cases on hemodialysis 2 sessions weekly (1790.7± 2062.7 pg/ml) was higher than those on hemodialysis 3 sessions weekly (1667.5± 1458.2 pg/ml) as shown in **Table 4.6**.

Table 4.6. Averages of parathormone, Ca×P product, albumin- corrected serum calcium, and serum phosphorus among cases by frequency of hemodialysis

Variable	Hemodialysis frequency		P-value
	Two sessions (n = 31)	Three sessions (n = 49)	
	Mean \pm SD	Mean \pm SD	
PTH (pg/ml)	1790.7 \pm 2062.7	1667.5 \pm 1458.2	0.755
Ca \times P (mg ² /dl ²)	61.6 \pm 15.7	63.4 \pm 14.0	0.592
Albumin - corrected serum Ca (mg/dl)	9.5 \pm 0.8	9.5 \pm 1.0	0.963
P(mg/dl)	6.5 \pm 1.7	6.7 \pm 1.3	0.611

4.4. Relationship between parathormone with albumin- corrected serum calcium, and with phosphorus among cases

The correlation between the parathormone with albumin-corrected serum calcium was statistically not significant (R= -0.20, P=0.08) among cases. Also the correlation between the parathormone with serum phosphorus was statistically not significance (R=0.21, P=0.060) among cases, where the increasing in the level of serum phosphorus results in increasing the level of parathormone.

4.5. Relationship between Vitamin D analogue, (alfacalcidol), with parathormone, calcium \times phosphorus product, albumin- corrected serum calcium and serum phosphorus among cases

Regarding the supplement of cases with Vitamin D analogue capsule of 0.25 microgram twice daily, the results revealed that there were no significant differences between the averages of parathormone, calcium \times phosphorus product, albumin-corrected serum calcium and serum phosphorus in cases supplied by vitamin D analogue (1749.0 \pm 1907.6pg/ml, 61.9 \pm 13.8 mg²/dl², 9.4 \pm 0.8 mg/dl and 6.6 \pm 1.4 mg/dl respectively) and those not supplied by vitamin D analogue (1641.0 \pm 1177.7pg/ml, 64.4 \pm 16.3 mg²/dl², 9.6 \pm 1.0 mg/dl and 6.7 \pm 1.4 mg/dl respectively) as shown in **Table 4.7**.

Table 4.7. Averages of parathormone, Ca×P product, albumin- corrected serum calcium and serum phosphorus among cases by vitamin D analogue supplying

Variable	Supplied with Vitamin D analogue (0.5 µg) daily. (n = 55)	Not Supplied with Vitamin D analogue (n = 25)	P-value
	Mean \pm SD	Mean \pm SD	
PTH (pg/ml)	1749.0 \pm 1907.6	1641.0 \pm 1177.7	0.795
Ca \times P (mg ² /dl ²)	61.9 \pm 13.8	64.4 \pm 16.3	0.480
Albumin - corrected serum Ca (mg/dl)	9.4 \pm 0.8	9.6 \pm 1.0	0.381
P(mg/dl)	6.6 \pm 1.4	6.7 \pm 1.4	0.829

4.6. Relationship between parathormone and calcium× phosphorus product with serum urea and creatinine among cases

Among the cases, it was found that there were no statistically significant correlations between parathormone with either serum urea (R=0.17, P=0.129) or creatinine (R=0.09, P=0.432). Also the correlation between (Ca \times P) product with serum urea was statistically not significant (R=0.12, P=0.280). On the other hand the correlation between (Ca \times P) product with creatinine was statistically significant (R=0.26, P=0.021), where increasing the level of serum creatinine results in increasing (Ca \times P) product according the formula ((Ca \times P) product = 52.26 +{1.55 \times Creatinine}) as shown in figure 4.10. Moreover, the correlation between serum phosphorus and creatinine was statistically significant (R=0.30, P=0.007), where the increasing in the level of serum creatinine results in increasing in the level of serum phosphorus according to the formula (serum phosphorus= 5.41 +{0.18 \times Creatinine }) as shown in figure 4.11.

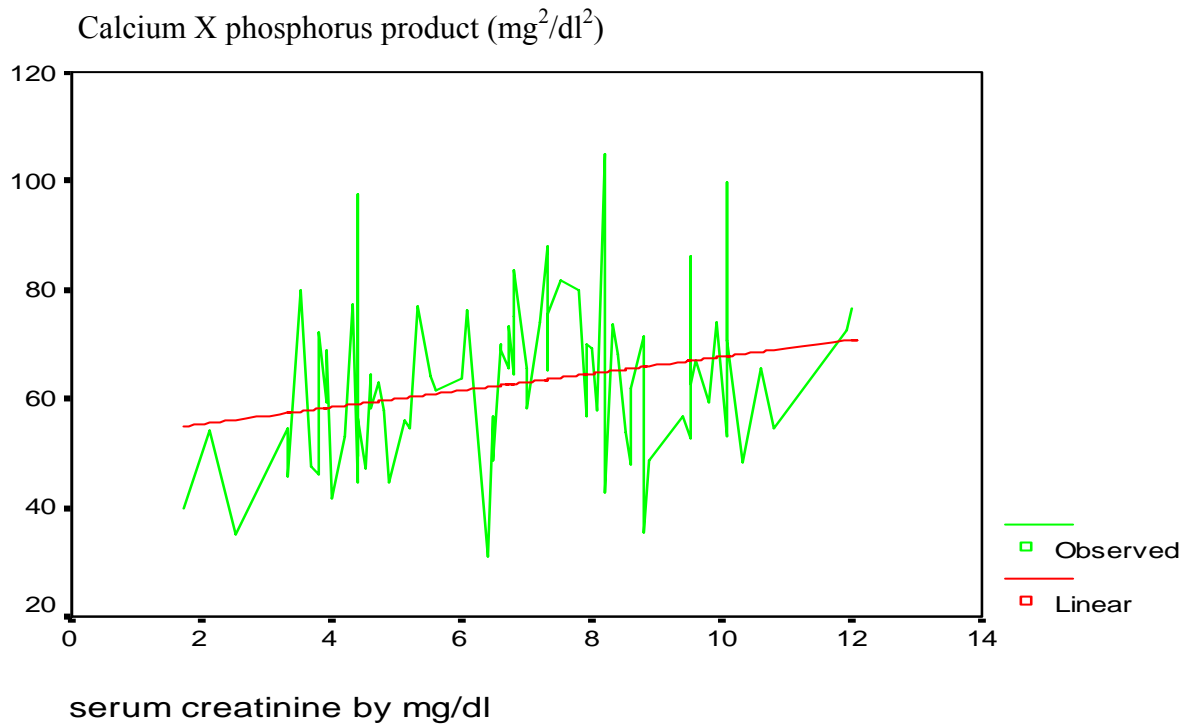


Figure 4.10. Correlation between calcium×phosphorus product with creatinine

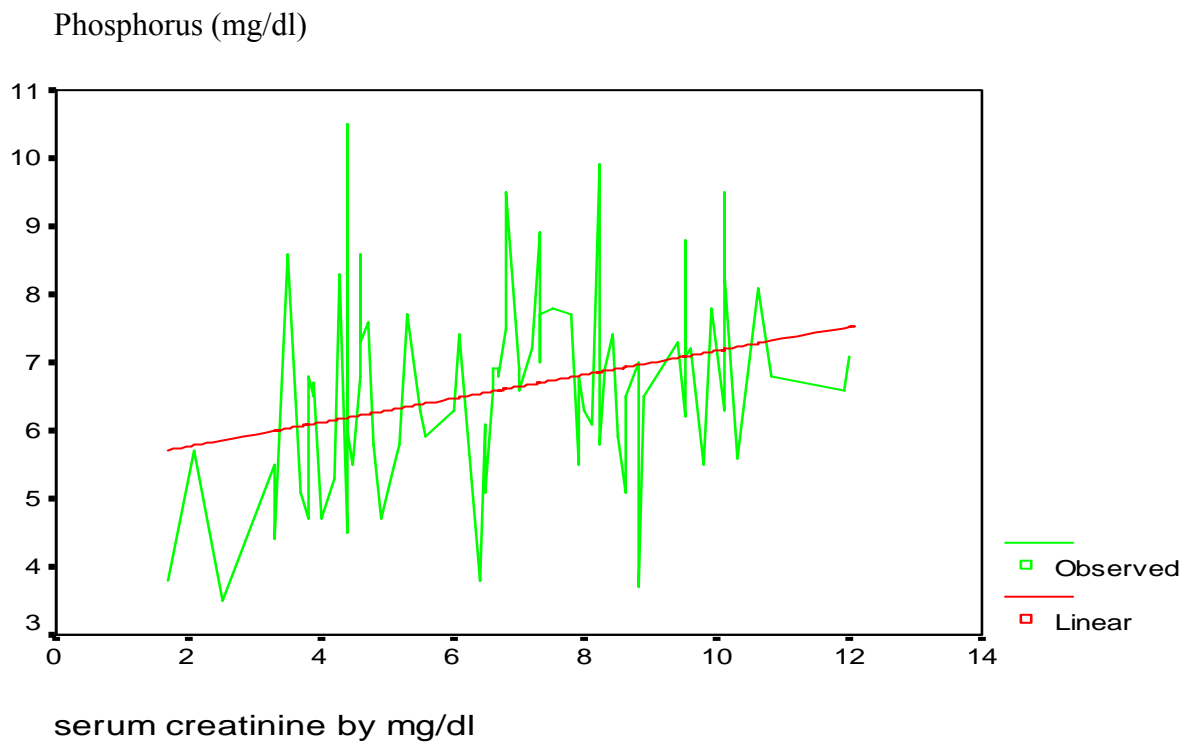


Figure 4.11. Correlation between serum phosphorus with creatinine

4.7. Relationship between parathormone and calcium× phosphorus product with age of cases

Regarding the age of cases, it was found that there was no correlation between PTH ($R = -0.14$, $p = 0.218$), or $(Ca \times P)$ product ($R = -0.017$, $p = 0.882$) with age.

4.8. Relationship between parathormone and calcium× phosphorus product with BMI of cases

It was found that there was no correlations between PTH ($R = 0.049$, $p = 0.665$) or $(Ca \times P)$ product ($R = 0.21$, $p = 0.062$) with BMI.

4.9. relationship of hemodialysis with bone and cardiovascular diseases among cases

The results of the study revealed that 37 (46.3%) subjects of 80 cases were complaining of bone diseases. It was observed that the percentage of subjects of bone diseases among cases on hemodialysis for less than 48 months 29 (59.2%) was more than those on hemodialysis for 48 months or more 8 (25.8%) as shown in **Figure 4.12**. Also the results revealed that there was a significant relationship between the duration of hemodialysis and bone diseases ($X^2 = 8.51$, $P = 0.004$). On the other hand there was no difference in the percentage of subjects of bone diseases among cases on hemodialysis of 2 sessions weekly 14 (45.2%) or those of three sessions weekly 23 (46.9%) **Figure 4.13**.

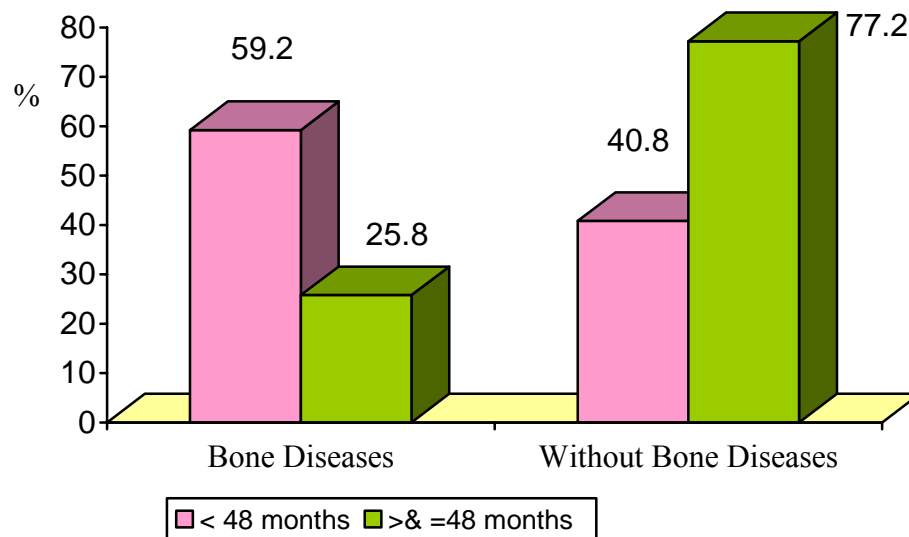


Figure 4.12. Distribution of cases by bone diseases and duration of hemodialysis

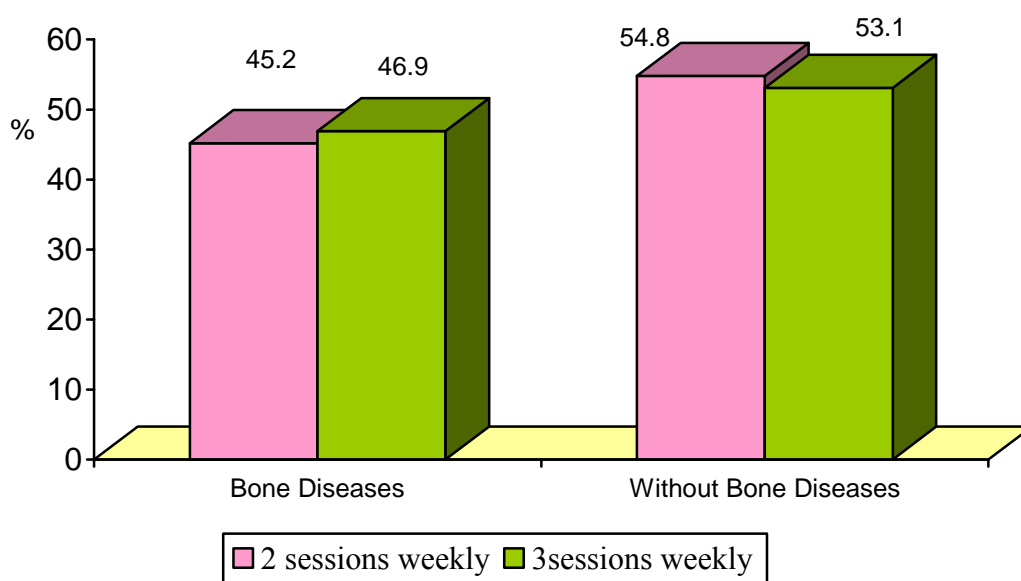


Figure 4.13. Distribution of cases by bone diseases and frequency of hemodialysis

Concerning cardiovascular disorders, the results revealed that the percentage of subjects of cardiovascular diseases among cases on hemodialysis for less than 48 months 12 (24.5%) was more than those on hemodialysis for 48 months or more 6 (19.4%), **Figure 4.14**. This difference was not statistically significant ($X^2= 0.29$, $P= 0.59$). However, there was statistically significant difference ($X^2= 7.48$, $P= 0.006$) in the

percentage of cases on hemodialysis two sessions weekly 2 (6.5%) and those on hemodialysis three sessions weekly 16 (32.7%), **Figure 4.15.**

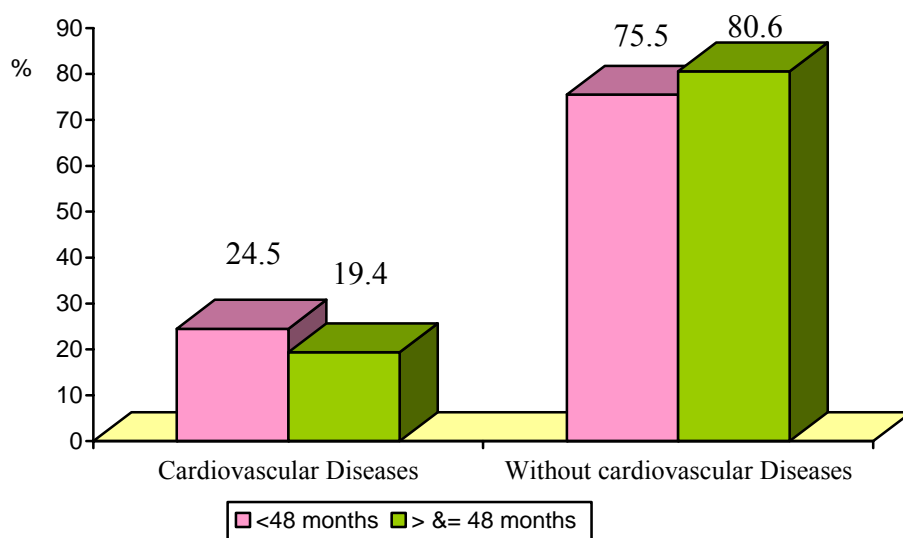


Figure 4.14. Distribution of cases by cardiovascular diseases and duration of hemodialysis

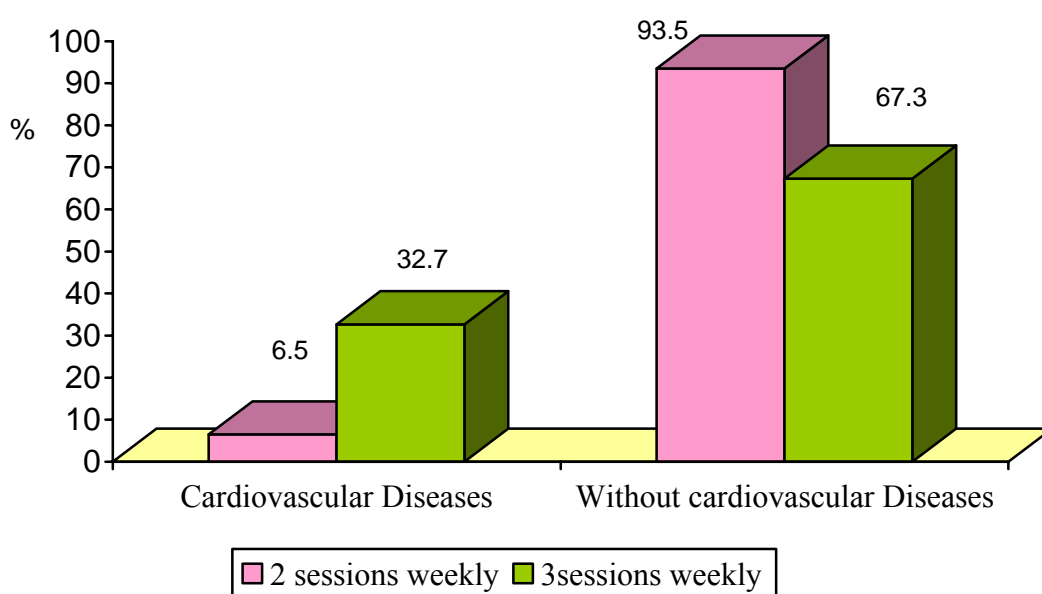


Figure 4.15. Distribution of cases by cardiovascular diseases and frequency of hemodialysis

Chapter 5

Discussion

Minerals are very important for the human body. They have various roles in metabolism and body functions and are essential for the proper function of cells, tissues and organs. Mineral metabolism disorders are marked by abnormal levels of minerals, either too much or too little, in the blood. Many people who have severe chronic kidney disease will eventually develop kidney failure and will require dialysis. Because of the importance of maintaining the levels of serum calcium, phosphorus and PTH to be in the recommended range described by K/DOQI guidelines in HD patients, we conduct this study to assess their levels.

Eighty HD patients in the HD unit at Al-Shifa hospital (41 male, 39 female; mean age 47.2 ± 15.9 years) on HD for ≥ 12 months (mean \pm SD: 49.1 ± 38 months, range: 12–163 months) with mean HD frequency (2.6 ± 0.5 sessions weekly), were included in the study. All patients were on phosphate binder (calcium carbonate), while 55 patients were receiving vitamin D analogue ($0.5 \mu\text{g}$ alfacalcidol daily), and the other 25 patients were not. Age and sex matched healthy control group were also included in the study.

5.1. Relationship between parathormone and calcium \times phosphorus product, albumin- corrected serum calcium and serum phosphorus with gender among cases and controls

It was shown that there were no statistically significant differences between males and females with regard to PTH, calcium \times phosphate product, albumin-corrected serum calcium and phosphorus levels in case and control groups, which means that gender has no effect on the concentration of these parameters. These results are congruent with others who did not find any statistically significant differences between serum PTH, Ca \times P products, serum total calcium and serum phosphorus, between males and females [107]. This may be explained by the fact that PTH secretion is not controlled by any other endocrine gland [18]. The major regulator of PTH secretion is the concentration of ionized calcium in blood, where PTH levels increase in response to decreased serum calcium and decrease in response to increased serum

calcium resulting in a close inverse relationship between PTH and calcium concentrations [108,109]. Also, high phosphate enhances parathyroid cell proliferation and PTH synthesis and secretion [110].

5.2. Levels of parathormone, calcium× phosphorus product, serum albumin, albumin- corrected serum calcium, and serum phosphorus among cases and controls

The study showed statistically significant differences between case and control groups with regard to PTH, calcium × phosphate product and phosphorus, where their mean values were higher in cases, and serum albumin and ionized calcium, where their mean values were lower in cases. It was shown that there was no statistically significant difference between the two groups with regard to the mean of albumin-corrected serum calcium, where the values were in the recommended reference range, where it was found that with initiation of regular HD, the levels of serum total calcium usually normalize [111]. In advanced stages of CKD, the fraction of total calcium bound to complexes was increased [112], thus, free (ionized) calcium levels were decreased despite normal total serum calcium levels.

Also, it was reported that impaired phosphate excretion, with the resulting hyperphosphatemia, is one of the earliest consequences of chronic renal failure. Hyperphosphatemia in turn plays an important role in the development of secondary hyperparathyroidism [113,114]. Moreover, phosphate retention leads to a decrease in serum free calcium levels, which in turn stimulates PTH secretion [115].

Although, the value of serum albumin of the patients is in the accepted reference range, which indicate that the patients are maintained in a good nutrition state, as serum albumin levels have been used extensively to assess the nutritional status of individuals with and without chronic renal failure [116], the value of serum albumin in the case group was less than that of the control group. It is known that about 6–10 g of amino acids is lost into the dialyzate during one session of HD with a low-flux membrane, and a loss of 1–2 g of albumin can be added if a high-flux membrane is used [117].

Concerning K/DOQI guidelines for mineral metabolism values in HD patients, the study revealed that 13.8%, 32.5%, 41.3% and 22.5% of the hemodialysis patients were in the range recommended by K/DOQI guidelines for PTH, $\text{Ca} \times \text{P}$ product, albumin-corrected serum calcium and serum phosphorus respectively. Only, 2.5% of the HD patients were within range for all the previously mentioned parameters. These results indicate that most of the HD patients are away from the recommended range. The percentage of patients, who were in the recommended range, were less than that obtained in other studies [2, 102, 105], only, calcium levels were comparable to that obtained in (DOPPS I and DOPPS II) [104]. The explanation of these differences may be attributed to implementing different modalities in regulation of phosphorus and PTH in those countries, as using other phosphate binders as, sevelamer hydrochloride, which is widely available in the USA and Europe for the treatment of hyperphosphatemia in patients with CKD [118] or lanthanum carbonate, which is the most recent non-calcium, non-aluminium phosphate binder to be developed for the treatment of hyperphosphatemia [119]. In addition, other vitamin D analogues as, 22-oxacalcitriol [120], paricalcitol [121], or doxercalciferol [122], may be used for controlling PTH secretion with minimal hypercalcemia and hyperphosphatemia compared to calcitriol. Paricalcitol and doxercalciferol are currently available for clinical use in the USA [123]. Also, using of cinacalcet HCl, which is a new calcimimetic agent that act at the level of the CaR. Activation of this receptor by calcimimetics increases intracellular calcium concentration, which causes rapid reduction in PTH secretion, serum phosphorus levels and the $\text{Ca} \times \text{P}$ product, which remain suppressed for up to three years [124].

The inability of the HD unit at Al-Shifa hospital in achieving a high percentage of patients in the range may be explained by many factors. Firstly, using calcium carbonate as a phosphate binder only. Moreover, the dose of calcium carbonate used may be less than that required for optimal effect. Secondly, using daily oral doses (0.5 μg) of the vitamin D analogue, alfacalcidol only for controlling PTH secretion, while most HD patients in the USA are now managed with thrice-weekly intravenous doses of calcitriol or other vitamin D analogues [123].

On the other hand, there were (42 patients, 52.5%) and (22 patients, 27.5%) receiving alfacalcidol, while their serum phosphorus and albumin- corrected serum

calcium were more than 5.5 mg/dl and more than 9.5 mg/dl, respectively. According to K/DOQI guidelines, alfacalcidol, should be discontinued when PTH levels decrease below target levels, or if calcium or phosphate levels increase above target levels. Moreover, parathyroidectomy, (subtotal or total), was not performed to the HD patients whose PTH values were more than 800 pg/ml (n=54, 67.5%) as indicated by K/DOQI guidelines; parathyroidectomy should be recommended in patients with severe hyperparathyroidism (persistent serum levels of intact PTH >800 pg/mL, associated with hypercalcemia and/or hyperphosphatemia that are refractory to medical therapy [125]. It was found that parathyroidectomy rates in U.S. HD patients increased between 1998 and 2002. The annual incidence of parathyroidectomy was 6.8 per 1000 patient-years in 1998 but, the rates increased progressively after 1998, reaching 11.8 per 1000 patient-years in 2002[126].

5.3.Averages of parathormone, (Calcium × phosphate) product, albumin-corrected serum calcium and serum phosphorus among cases by duration of hemodialysis

In studying the effect of duration of HD on the levels of PTH, Ca × P product, albumin-corrected serum calcium and phosphorus, it was found that there were no statistically significant differences in the levels of Ca × P product, albumin-corrected serum calcium and phosphorus in the group on HD for < 48 months and the group on HD for ≥ 48 months. Conversely, the difference in PTH levels was statistically significant, where it was higher in the group on HD for ≥ 48 months, which indicates that the parathyroid glands activity was in a positive correlation with the duration of HD. This result is supported by other study that found a significant positive correlation of serum PTH with HD duration [107]. Also, an increase in PTH was found with time on dialysis, an increase that is significant even after adjusting for calcium and phosphorus concentration [127]. The explanation of this result may be due to that the parathyroid glands were more activated due to the continuous state of stimulation due to persistent hyperphosphatemia and consequently, low concentration of ionized calcium. High phosphate enhances parathyroid cell proliferation and PTH synthesis and secretion directly and indirectly through both a reduction in serum calcitriol and ionized calcium levels [110].

5.4. Averages of parathormone, (Calcium \times phosphate) product, albumin-corrected serum calcium and serum phosphorus among cases by frequency of hemodialysis

Concerning the frequency of HD, the results of the study revealed that there were no statistically significant differences between the averages of PTH, Ca \times P product, albumin-corrected serum calcium and serum phosphorus of cases on HD 2 sessions weekly and of those on HD 3 sessions weekly. It was observed that the values of PTH, Ca \times P product and serum phosphorus were highly elevated in both groups, and both were away from the recommended ranges. Only, albumin-corrected serum calcium levels were in the range.

It seems that both of 2 sessions or 3 sessions a week were inadequate for maintaining the levels of PTH, Ca \times P product and serum phosphorus to be in the recommended ranges. Results from clinical trials using daily HD strongly suggest that thrice-weekly HD regimens are only marginally adequate for achieving weekly phosphorus balance in many patients with ESRD [123]. Because of the kinetics of phosphate, increasing the frequency of dialysis sessions more effectively removes phosphate than increasing time of individual dialysis sessions [128,129]. Alternative dialysis regimens, such as daily nocturnal HD and short-duration HD done 6 days per week, provide much better control of serum phosphorus concentrations than conventional thrice-weekly HD [130]. It was reported that the results of this approach are sufficiently striking that it has been described as “dialysis for the next century” [131].

5.5. Relationship between parathormone with albumin-corrected serum calcium, and with phosphorus among cases

The study revealed that the correlation between the PTH with albumin-corrected serum calcium among cases was weak, inverse and statistically not significant. This result is similar to that obtained by others who demonstrated that serum PTH was not related to total serum calcium [132]. Also, some of the recent studies [133] did not find a relationship between elevated serum levels of PTH observed in CKD patients with

different levels of GFR and the levels of serum calcium, which were within the normal range independent of the stage of kidney disease.

Also, the present study revealed that the correlation between PTH with serum phosphorus among cases was weak, positive and at the border of statistical significance. This result is close to that obtained by others, who demonstrated that serum PTH was positively correlated with serum phosphorus [132]. It was found that PTH did not correlate with total calcium, and the correlation between PTH and serum phosphorous was of borderline significance [134].

The weak correlation between serum PTH and serum phosphorus may denotes that the increase in serum phosphorus results in the increase of PTH levels among cases, but the effect of phosphorus on the parathyroid glands is weak, which may suggest that some patients may had tertiary hyperparathyroidism.

5.6. Relationship between vitamin D analogue, (alfacalcidol), with parathormone, calcium× phosphorus product, albumin- corrected serum calcium and serum phosphorus among cases

It was shown in the study that there were no statistically significant differences between the averages of PTH, $\text{Ca} \times \text{P}$ product, albumin-corrected serum calcium and serum phosphorus of cases supplied with oral vitamin D analogue (alfacalcidol, 0.5µg) daily and those not supplied with it. It was observed that the values of PTH, $\text{Ca} \times \text{P}$ product and serum phosphorus were highly elevated in both groups, and both were away of the recommended ranges. Only, albumin-corrected serum calcium levels were close to the range.

It seems that the use of, alfacalcidol, for controlling PTH levels in hemodialysis patients is inadequate, may be, the dose is low , as the K/DOQI recommended doses of calcitriol ranges from 0.5 to 7.0 µg per HD session depending on the levels of PTH, calcium and phosphorus in the serum of HD patients. The dose given to the patients, may be, just sufficient to control serum calcium levels, but insufficient to control PTH levels. It was found that up to half of patients with sever secondary hyperparathyroidism showed little or no decline in plasma PTH levels with vitamin D therapy [135]

Also, a resistance to the action of the drug on the parathyroid glands, caused by persistent hyperphosphatemia may be developed, where hyperphosphatemia is associated with resistance to the actions of calcitriol on the parathyroid glands, which leads to increased PTH secretion [136]

5.7. Relationship between parathormone and calcium \times phosphorus product with serum urea and creatinine among cases.

The study showed no statistically significant correlation between PTH with either serum urea or creatinine which indicates that the secretion of the PTH is independent of the effect of serum urea or creatinine, as mentioned previously, that the major regulator of PTH secretion is the concentration of ionized calcium in blood.

It was shown, also, that the correlation between Ca \times P product with serum urea was not statistically significant, while the correlation with serum creatinine was statistically significant. This, may be attributed to the HD process itself. According to the laws of diffusion, the larger the molecule, the slower its rate of transfer across the membrane of the HD machine. A small molecule such as urea (60 Da) undergoes substantial clearance, whereas a larger molecule such as creatinine (113 Da) is cleared less efficiently [83].

With respect to phosphate, during HD, serum phosphate drops rapidly in the first one to two hours of the treatment and then it reaches a plateau. The amount of phosphate removed decreases significantly in the second half of the dialysis. It has also been well documented that serum phosphate concentration rises relatively quickly in the first few hours after termination of dialysis (rebound phenomenon) [137,138]. So, both serum creatinine and serum phosphate remain relatively high despite the HD, and the correlation between them was statistically significant as shown in the study.

5.8. Relationship between parathormone and calcium × phosphate product with age of cases

The study revealed that there were no correlation between PTH or calcium × phosphate product with age. These results do not agree with others who found inverse correlations of age with serum PTH and serum phosphorus [107]. Conversely, an increase of PTH levels with age was reported in some studies [139]. These differences may be attributed to differences in the HD regimens applied.

5.9. Relationship between parathormone and calcium × phosphate product with BMI of cases

The study revealed that the average of BMI was $25.5 \pm 6.7 \text{ kg/m}^2$ among cases, which suggest that most of the HD patient were in a good nutrition state, and this was confirmed earlier as indicated by the level of serum albumin.

The value of BMI recorded was higher than that recorded in a group on HD who were classified as having severe secondary hyperparathyroidism, where it was $23 \pm 5.5 \text{ kg/m}^2$ [134].

BMI is an anthropometric measure frequently used to assess nutritional status in HD patients. ESRD patients treated with HD with higher BMI have increased survival over a 1-year period [140-144]. In the general population, patients with lower BMI usually have increased survival [140,141,145]. Further research is needed in this area to explain the reasons for the differences between the findings in the general population and ESRD patients. It was found that HD patients with BMI between 25 and 29.9 had the best survival [146]. Also, the study revealed that there was no correlation between PTH with BMI, but the correlation between calcium × phosphate product with BMI was at the border of statistical significance. Our results differ from that obtained in a population based, cross- sectional study, where it was found that, for serum calcium and PTH there was a significant positive relation to BMI in both genders [147]. However, it was found that there was no association between BMI and calcium intake in women, whereas a positive and significant association was found in men[148]. The differences may be attributed to the severity of hyperparathyroidism encountered in some of our patients.

5.10. Relationship of hemodialysis with bone and cardiovascular diseases among cases

The study revealed that 37 (46.3%) and 18 (22.5%) of the HD patients were complaining of bone and cardiovascular diseases, respectively. It was shown that PTH is a major uremic toxin [149,150], and may be responsible for long-term consequences that include renal osteodystrophy, severe vascular calcification, alterations in cardiovascular structure and function, immune dysfunction, and anemia. These adverse effects may contribute to an increased risk of cardiovascular morbidity and mortality among ESRD patients [149-152].

The study revealed that there were a significant relationship between the duration of HD and bone diseases. Renal bone disease starts early in the course of chronic renal failure and progresses as the kidney function deteriorates. In the immediate predialysis period, almost all patients have abnormal bone histologies [153]. Bone disease in patients with chronic renal failure is usually asymptomatic, thus symptoms appear late in the course of renal osteodystrophy [154].

It was shown in the study, that there was no statistically significant difference of the percentage of HD patients who are on hemodialysis 2 sessions weekly and those on hemodialysis 3 sessions weekly. This may be due to establishment of bone disease long before starting dialysis treatment. Bone derangement and vascular calcifications are difficult to reverse when established, which mandates early management of secondary hyperparathyroidism and consideration of factors involved in the activity of the parathyroid glands [150]

Considering the cardiovascular disease, the study revealed that the percentage of HD patients complaining of cardiovascular diseases on HD for < 48 months (24.5%) were more than those on HD for \geq 48 months (19.4%), but the difference was not statistically significant. This difference may be attributed to increased mortality with the increase in the duration of HD. Cardiovascular disease accounts for more than 50% of deaths among persons with ESRD, and the annual cardiovascular mortality rate is more

than an order of magnitude greater than in the non-ESRD population, especially among younger (<70 years) individuals [155].

Recent evidence has shown a high prevalence of coronary artery calcifications in the ESRD population, and they probably play a major role in the high cardiac morbidity and mortality rates. Coronary artery calcifications are much more common and more severe in patients on HD than in subjects without renal failure [156,157]. Most studies have found correlations of calcifications with uncontrolled hyperphosphatemia, an increased calcium \times phosphate product and years on dialysis [158,159].

Also, the study revealed that there was a statistically significant difference ($P=0.006$) in the percentage of cases on HD 2 sessions weekly (6.5%), and those on HD 3 sessions weekly (32.7%). This difference may indicate, how much the increasing of HD sessions is important for the survival of HD patients. It is known that hyperphosphatemia is associated with development of hyperparathyroidism, renal osteodystrophy and increased morbidity and mortality [27]. So, controlling serum phosphorus is very important for the HD patients, and this was achieved with other modalities as increasing HD sessions. Indeed, most patients undergoing nightly HD maintain normal serum phosphorus concentrations without ongoing treatment with phosphate-binding agents [130].

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

- 1- K/DOQI guidelines for mineral metabolism in hemodialysis patients were satisfied in only a small proportion of patients.
- 2- The majority of hemodialysis patients have highly elevated serum PTH and phosphorus levels that suggest that some of the patients have severe secondary hyperparathyroidism or more severely, tertiary hyperparathyroidism.
- 3- There was a marked increase in serum PTH levels with the increase of hemodialysis duration.
- 4- There were no differences between the averages of PTH, calcium \times phosphate product, albumin-corrected serum calcium and serum phosphorus of patients on hemodialysis 2 sessions weekly and of those on hemodialysis 3 sessions weekly. Only, albumin-corrected serum calcium levels were in the range, while the others were highly elevated in both groups.
- 5- There were no differences between the averages of PTH, calcium \times phosphate product, albumin-corrected serum calcium and serum phosphorus of patients receiving oral vitamin D analogue (alfacalcidol, 0.5 μ g) daily and those not receiving it. Only, albumin-corrected serum calcium levels were close to the range, while the others were highly elevated in both groups.
- 6- There was no correlation between PTH with albumin-corrected serum calcium, but the correlation between PTH with serum phosphorus was at the border of statistical significance in the hemodialysis patients..
- 7- There were no correlations between PTH with either serum urea or creatinine. Also, there was no correlation between calcium \times phosphate product with serum urea, but the correlation with serum creatinine was statistically significant.
- 8- There were no correlations between PTH or calcium \times phosphate product with both of age or BMI of hemodialysis patients.
- 9- Many hemodialysis patients are complaining of bone and/or cardiovascular diseases. There was a statistically significant relationship between the duration of hemodialysis and bone disease.

- 10- The highly unsatisfactory results achieved in the study may be due to not implementing K/DOQI guidelines at HD unit, poor patient compliance and/or the ineffectiveness of available treatments.

6.2 Recommendations

- 1- Periodic monitoring of serum PTH ,calcium and phosphorus of the hemodialysis patients, and this require supplying the central laboratory with the necessary kits, particularly PTH kits.
- 2- Raising awareness of CKD and K/DOQI guidelines among renal physicians, the staff dealing with hemodialysis patients and the patients.
- 3- Conducting clinical trials to explore the impact of applying new methods and strategies of controlling calcium and phosphorus metabolism and of controlling parathyroid gland activity on the outcome of the CKD and hemodialysis patients.

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Appendices

Annex 1

Palestinian National Authority
Ministry of Health
Helsinki Committee



السلطة الوطنية الفلسطينية
وزارة الصحة
لجنة هلسنكي

Date: 5/3/2008

التاريخ: ٢٠٠٨/ ٣/ ٥

Name: Hosam Abo Shammala

الاسم: حسام أبو شمالة

I would like to inform you that the committee has discussed your application :

نفيدكم علما بان اللجنة قد ناقشت مقترح دراستكم حول:

Parathormone, Calcium, and
Phosphorous in Hemodialysis Patients at
Al-Shifa Hospital, palestine.

In it's meeting on _____ and
decided the following :-

و قد

و ذلك في جلستها المنعقدة لشهر
قررت ما يلي :

To approve the above mentioned
research study

25.3.2008

الموافقة على البحث المذكور عاليه



Signature
التوقيع

Acting Chairperson



Conditions:-

- Valid for two years from the date of approval to start
- It is necessary to notify the committee in any change in the admitted study protocol
- The committee appreciate receiving one copy of your final research when it is completed

Gaza Etwan - Telefax 972-7-2878166



كلية العلوم

الجامعة الإسلامية - غزة

The Islamic University of Gaza

مدير برنامج ماجستير العلوم الحياتية

التاريخ / 2007/11/17م

حفظه الله ،،،

مدير مجمع الشفاء الطبي

الأخ الدكتور/ د. حسن خلف

السلام عليكم ورحمة الله وبركاته .

الموضوع / تسهيل مهمة باحث

نود أن نعلم سيادتكم بان الطالب/ حسام محمود أبوشماله ، طالب في ماجستير

العلوم الحياتية - تخصص تحاليل طبية في الجامعة الإسلامية يقوم بإجراء

بحث بعنوان:

Parathormone, Calcium, and phosphorus in Hemodialysis patients at Al-Shifa Hospital – Gaza – Palestine.

والطالب يحتاج لإجراء البحث الحصول علي معلومات خاصة بالبحث.

لذا نرجو من سيادتكم تسهيل مهمة الطالب في الحصول علي المعلومات اللازمة

ولكم جزيل الشكر والتقدير ،،،

مدير برنامج ماجستير العلوم الحياتية
د. عبود ياسر القيشاوي



لبحثه .
دكتور/ حسن عاشور
أخصائي انف وأذن وعذرة
ترخيص 20/84
المكتب الحسابي
المختبر
11/11

Islamic University P. Box 108 Al-Rimal Gaza Palestine
Tel: (970/8) 2860700 Fax: (970/8) 2860700 2863552 e-mail: public@mail.iugaza.edu Web Site: www.iugaza.edu

الجامعة الإسلامية - غزة - الرمال ص.ب: 108 فلسطين



كلية العلوم

الجامعة الإسلامية - غزة

The Islamic University of Gaza

مدير برنامج ماجستير العلوم الحياتية

التاريخ/ 2007/11/17م

حفظه الله ،،،

مدير مستشفى بيت حانون

الأخ الدكتور/ د. جميل سليمان

السلام عليكم ورحمة الله وبركاته .

الموضوع / تسهيل مهمة باحث

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لبحثه.

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Islamic University P. Box 108-AI-Rimal Gaza Palestine

Tel:(970/8)2860700 Fax:(970/8)2860700 2863552 e-mail:public@mail.iugaza.edu Web Site:www.iugaza.edu

الجامعة الإسلامية - غزة - الرمال من ب: 108 فلسطين

